ORFLO Revolutionizing Life Science

Moxi Flow

An Innovative New Approach to Flow Cytometry

Training Guide

20131105

By: Don O'Neil

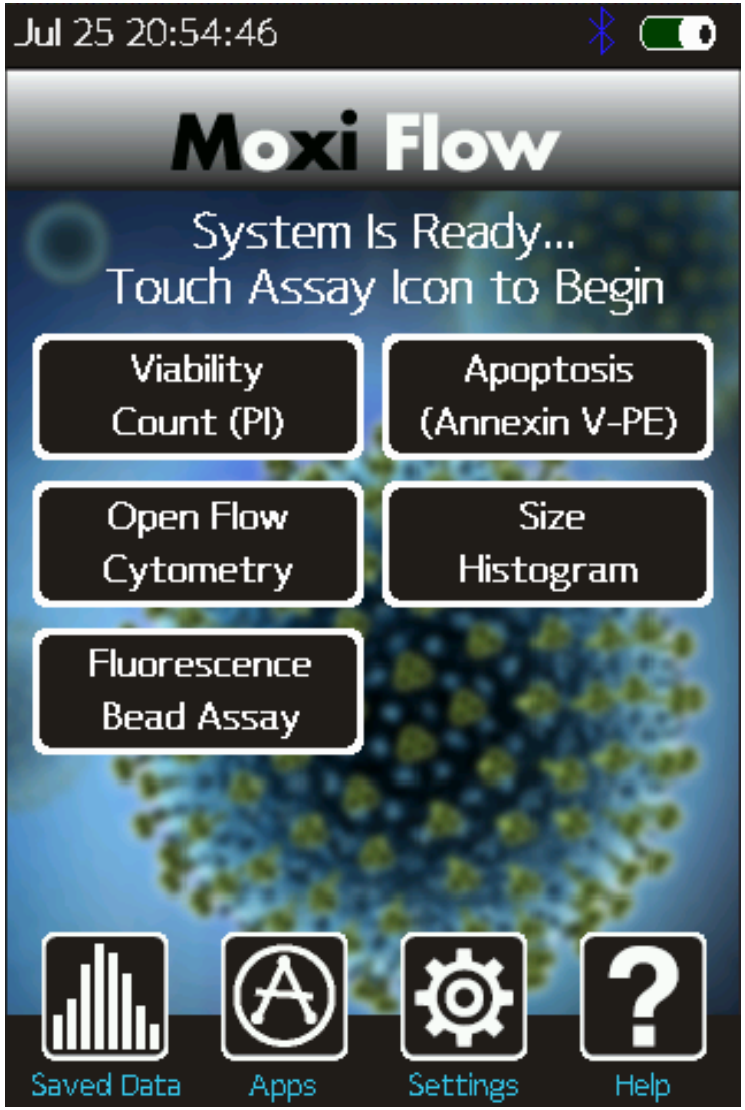
SVP, Sales and Strategic Partnership, ORFLO Technologies

A Flow Revolution.

- Amazing performance (up to 2.2 log separation)
- Combination 532 Laser w/ 590/40 PMT & Impedance detection (20nm size resolution
- No training
- 30 – 50K events measured in 10 seconds, outperforms any bench top or mid-tier flow cytometer on the market
- Brand new Flow Cell for every experiment
- Enables cell assay experiment "on-demand"
- Instant design of experiment execution & trend analysis
- Save thousands of dollars on waste, cleaning and down time issues associated with traditional flow cytometers
- Ideal for cancer, stem cell, immunology, metabolic disease research

The iPhone of Flow Cytometry

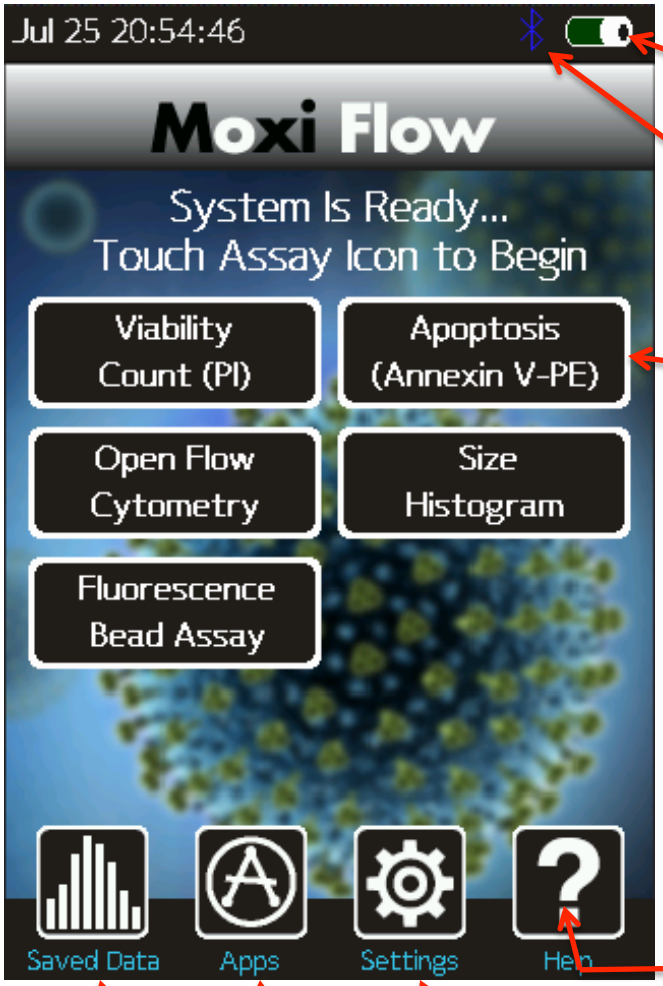
Intuitive User Interface



- Preset assays, no need for setting gains
  - Utilize "open-flow" for any PE labeled flow antibody
  - Compatible with PE, Sytox Orange, Ethidium Homodimer, Suncoast Yellow, Texas/Nile Red
- Ability to store 1000's of data files
- FCS 3.1 data output enables more complex analysis with standard flow software
- ORFLO's also offers VESTIGO, simple intuitive alternative to complicated flow analysis software
- Bluetooth label printing

Basic Functionality

Home Screen



Battery indicator, if red, charge over night with wall plug
Always run experiments with instrument plugged into the wall
Battery power should last all week

Bluetooth enabled for printing screen dumps and adding to lab book

- Pre-set assay categories
- Eliminates need for gain and power settings, simply select and the system will walk you through how to run complete the test
- When new applications are developed (ie Cell Cycle) Orflo will send out OS updates, similar to iPhone, simply plug the unit in and drag and drop the files on top of the “Moxi-Flow” which appears as a hard drive in on your file manager

Click for help, last screen will give you latest OS

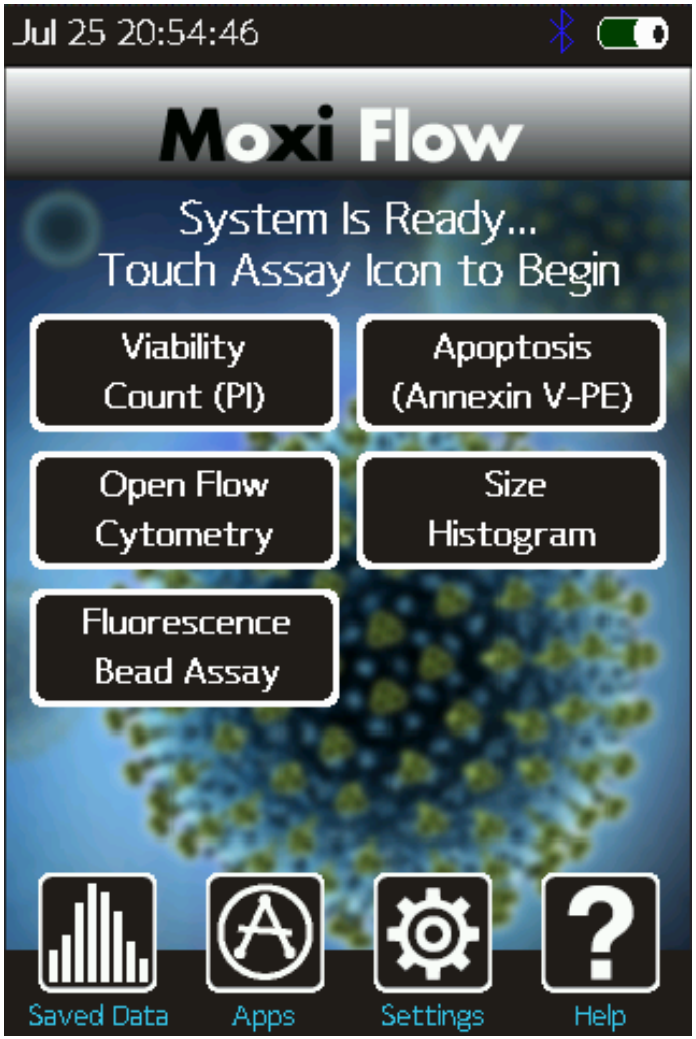
Click to set date and time

Click to view other basic apps, rarely used, not important to highlight

Click to view stored files (saves up to 500 data files on board)

Running An Experiment

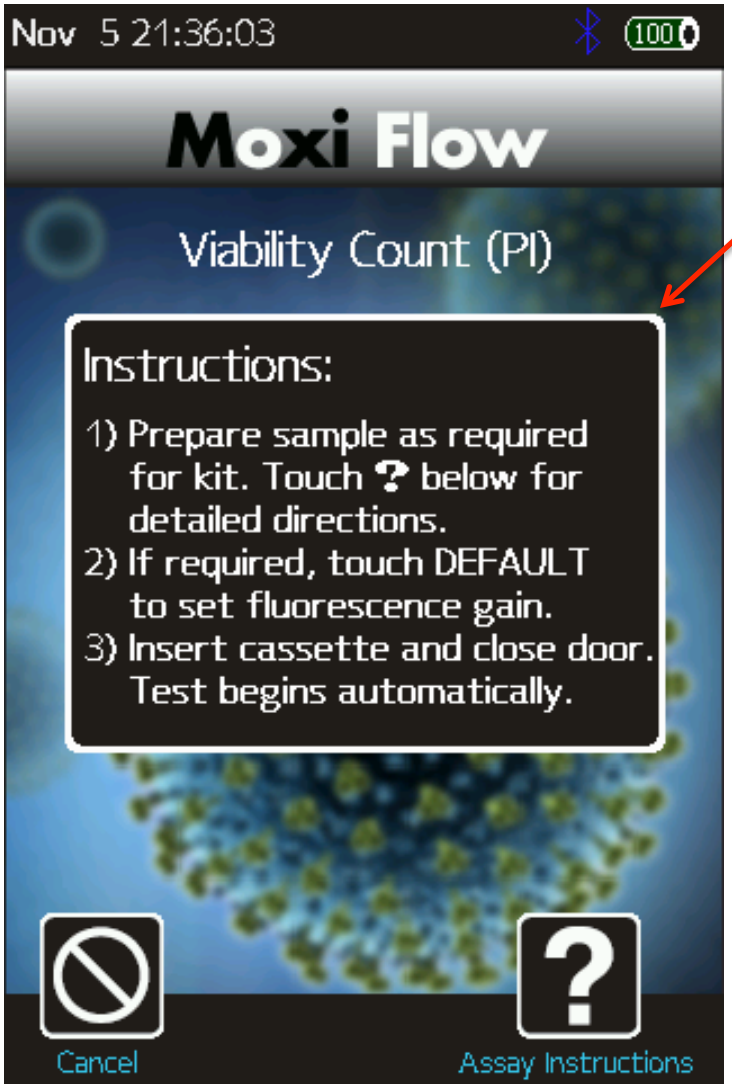
Select your desired assay



Select assay by touching desired box

Running An Experiment

Screen immediately after assay selection



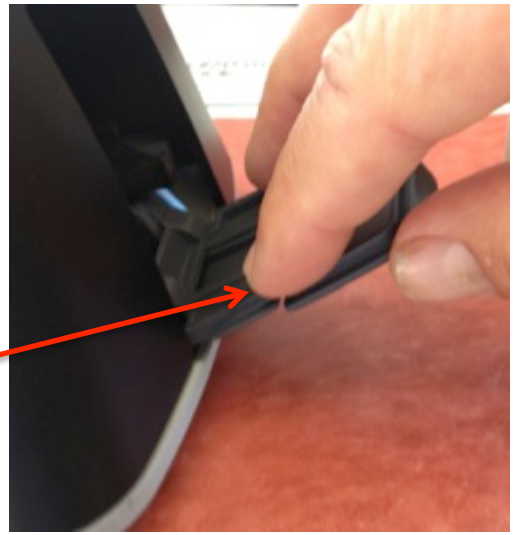
Step by step instructions given on screen

Running An Experiment

Load cassette

1. Open top and bottom doors of unit

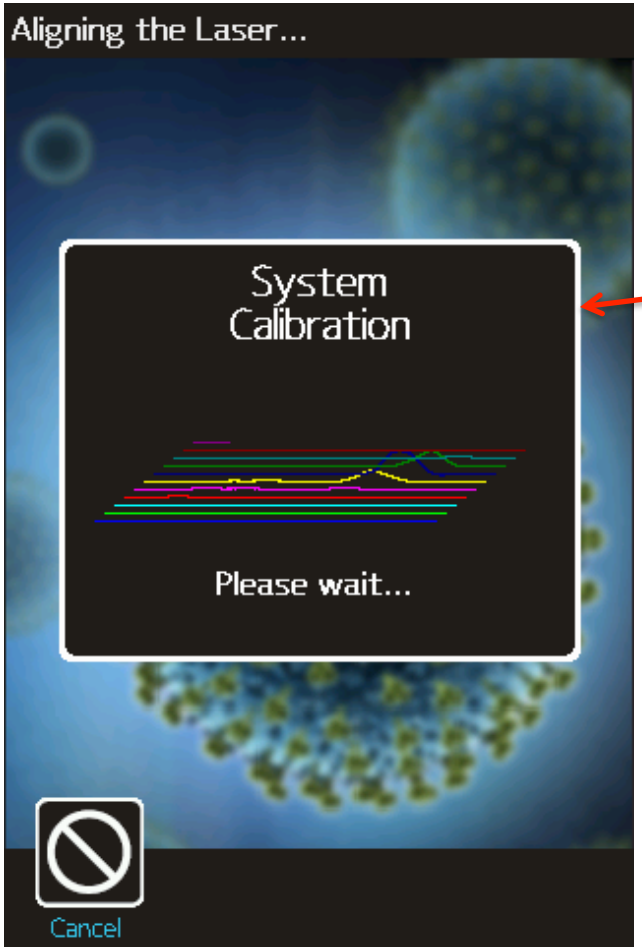
2. Insert the cassette



3. Close doors, be sure to keep index and pointer finger on lower door, while closing so that it doesn't slam shut
   - If door slams shut, it's not a problem, however closing the door gently is preferred

Running An Experiment

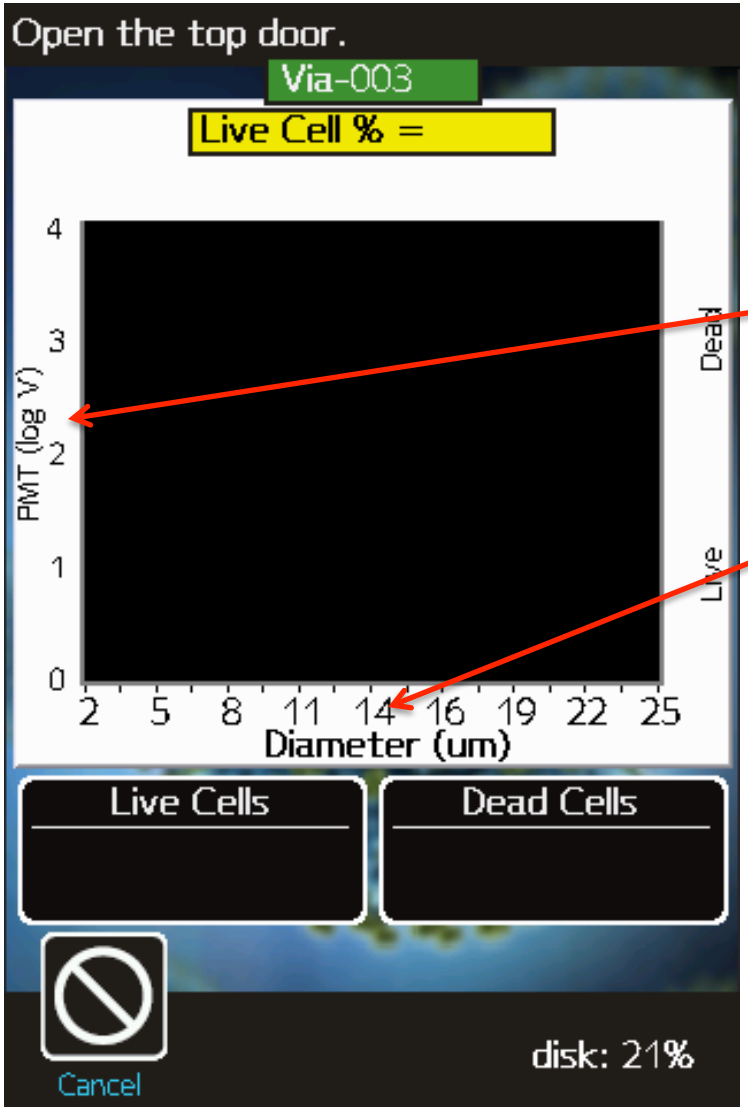
After inserting cassette & closing doors laser auto aligns



- Alignment takes about 15 seconds
- Do not open door until alignment completes
- This alignment eliminates a major issue associated with traditional flow cytometers that require lasers to be aligned, frequently after shipping and typically once per year, or more
    - Requires a service technician and translates to significant instrument down time

Running An Experiment

System is now ready for sample to be pipetted



- Scatter plot screen empty
- Data will plot in real-time once sample is pipetted and door is closed

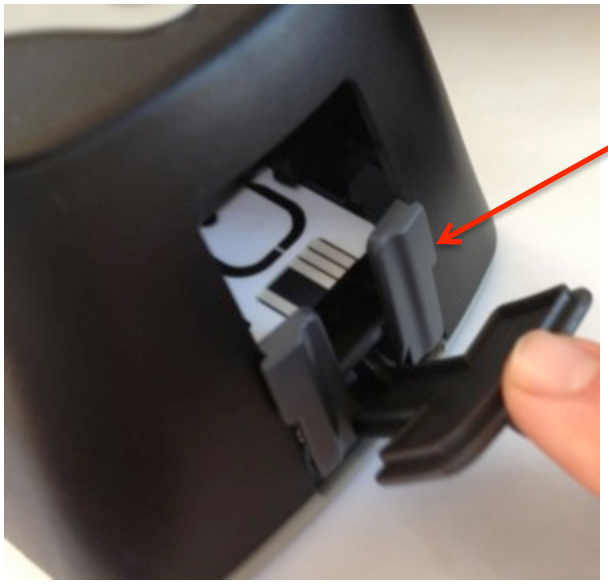
- Fluorescence intensity axis (voltage from PMT)
- Impedance axis, cell diameter

- Fluorescence intensity axis (voltage from PMT)
- Impedance axis, cell diameter

Running An Experiment

Pipette 5 sample into cassette

ORFLO Revolutionizing Life Science



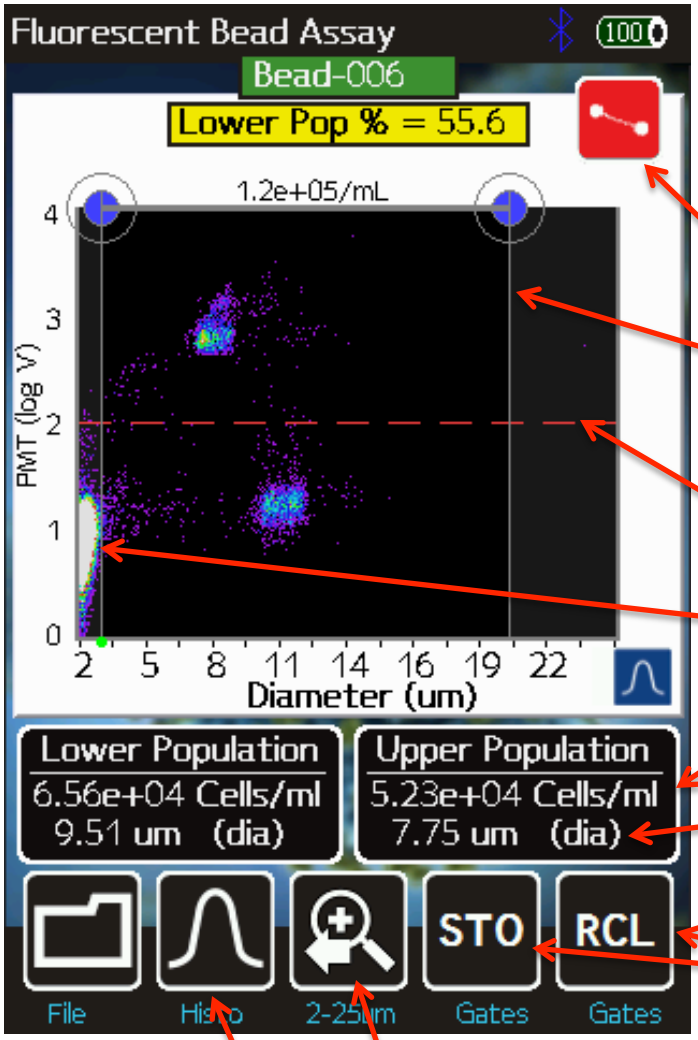
1. Open top door only

2. <u>Rapidly</u> pipette 50ul sample into well of cassette
- Do not worry about a bead forming
- It's important to pipette rapidly in order to avoid air being drawn in, which will compromise the test

3. Close doors, be sure to keep index and pointer finger on lower door, while closing so that it doesn't slam shut
4. The test will begin running and plot data in real-time

Basic Functionality

Manual Gate Mode



- Once complete data is presented in a scatter plot
- Brightness of each population indicates density/concentration
- The brighter the more concentrated the population

Icon for toggling between vertical and horizontal gate adjust

Vertical gate, use to separate two populations by their diameter, these gates are active note large blue circle at top of gate
Note green dot at bottom of left vertical gate, this keeps the gate perfectly vetical
Touch green dot and it will turn red, which enables you to angle gate

Horizontal gate, use to separate out non-stained and stained populations, gate is not active, vertical gates are in this screen shot

System noise

Mean cell concentration in gated region

Mean cell/volume in gated region

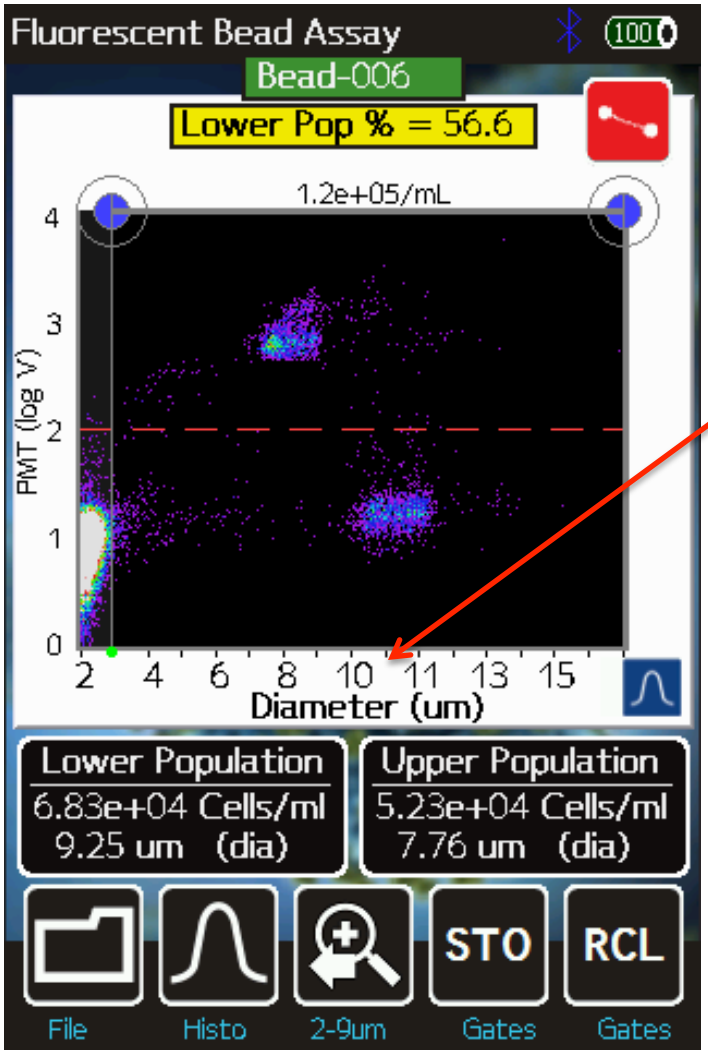
Store up to 5 gates settings, this is handy for blood samples that have multiple cell types, which can be gated by size

Use to zoom the x-axis (3 levels of auto scale)

Toggle between scatter plot and histogram view mode

Basic Functionality

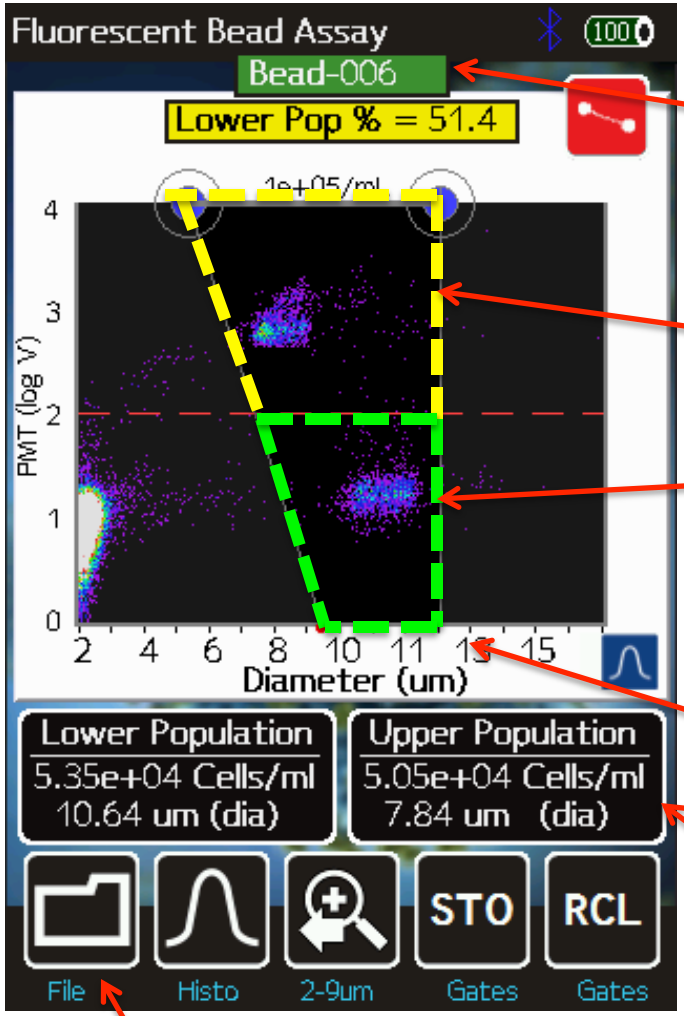
Manual Gate Mode



- X-axis zoomed in (2-17um)
- Notice populations look wider
- Next we will adjust the gates

Basic Functionality

Re-gated for accurate measurement



- File name, click to change
- If diluting, it may be handy to enter dilution factor into file name
- One proposed convention
  - "sample-name_cell-type_dilution-factor.fcs"

- These are the gated regions
- All values for fluorescence intensity and size will be generated from events only occurring in the these two regions
- Use the STO store gate function to gate around other populations and rapidly quantify their values

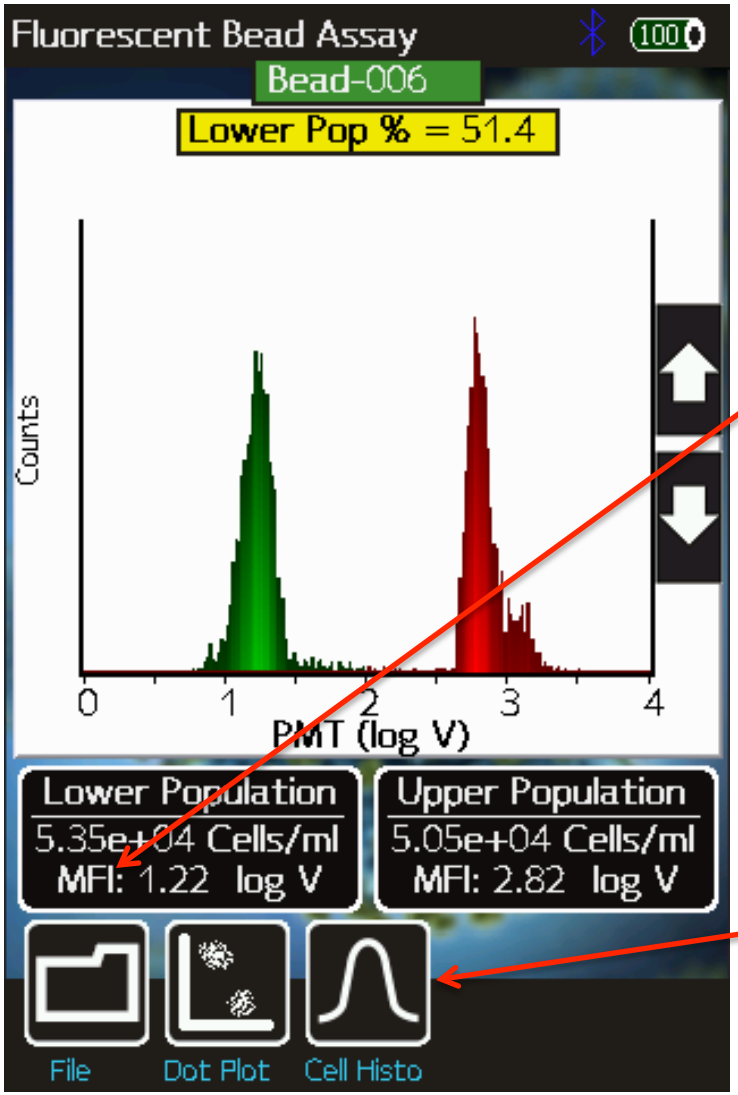
- Note left vertical gate has red dot at bottom
- This enables angling to gate
- The gate can be wrapped around the y-axis fluorescence, if a more extreme angle is required

- Note concentration and size have changed from the unadjusted gates

- Click to save and or print file
- Screen shot can be saved, or printed via blue-tooth label printer

Basic Functionality

Fluorescence histogram view



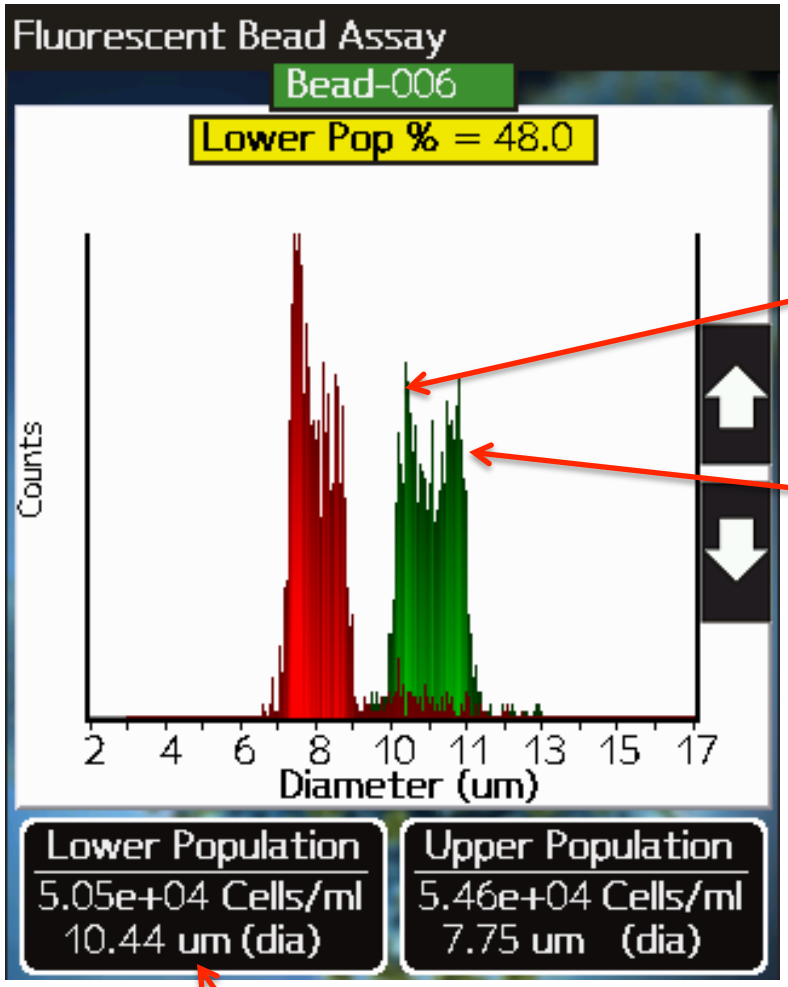
MFI: Mean fluorescence intensity, can be used to quantitate protein expression, based of user created standard curve

You can calculate the log difference in intensity between dim and stained population
Subtract MFI values: 2.82 – 1.22 = 1.6 log difference

Click “Cell Histo” icon to view size histogram

Basic Functionality

Cell diameter histogram view

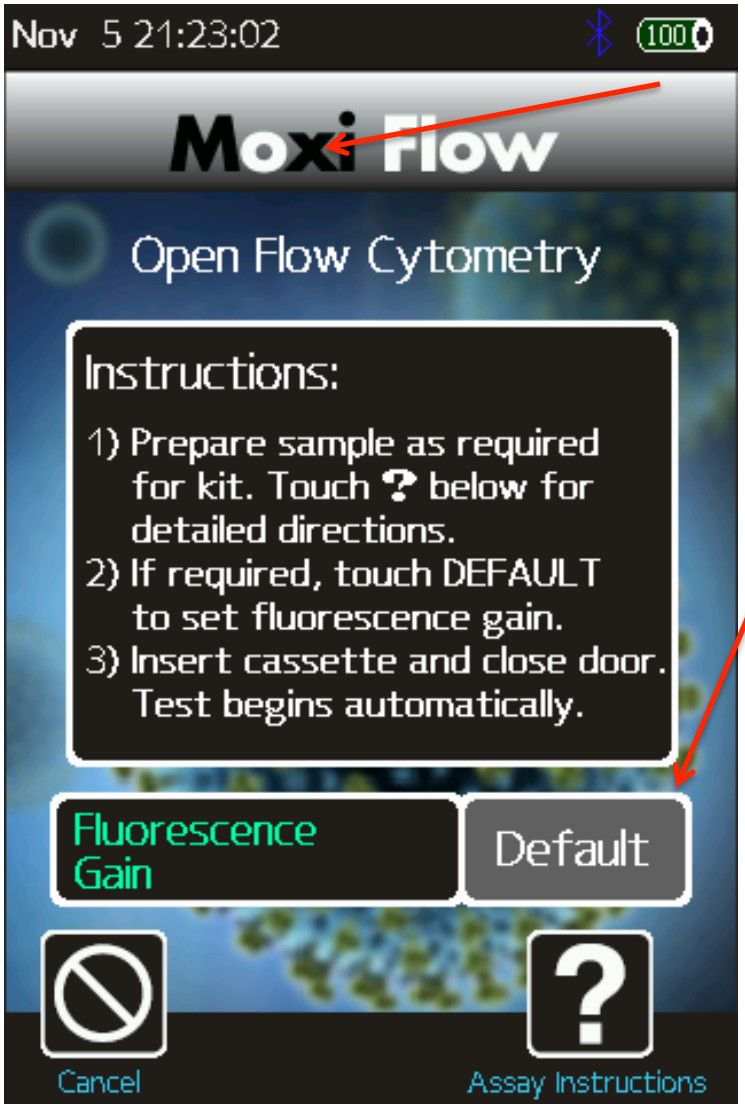


Observation:
Note both population are bi-modal in size
These are beads which are manufactured with two different bead sizes to achieve a desired mean diameter
The Moxi Flow with it's 10nm bin resolution has the sensitivity to detect these incredibly small size changes
This is an enabling feature for studying morphology changes (swelling) over time, that no other instrument can enable

Mean cell diameter of gated population intensity, can be used to quantitate protein expression, based of user created standard curve

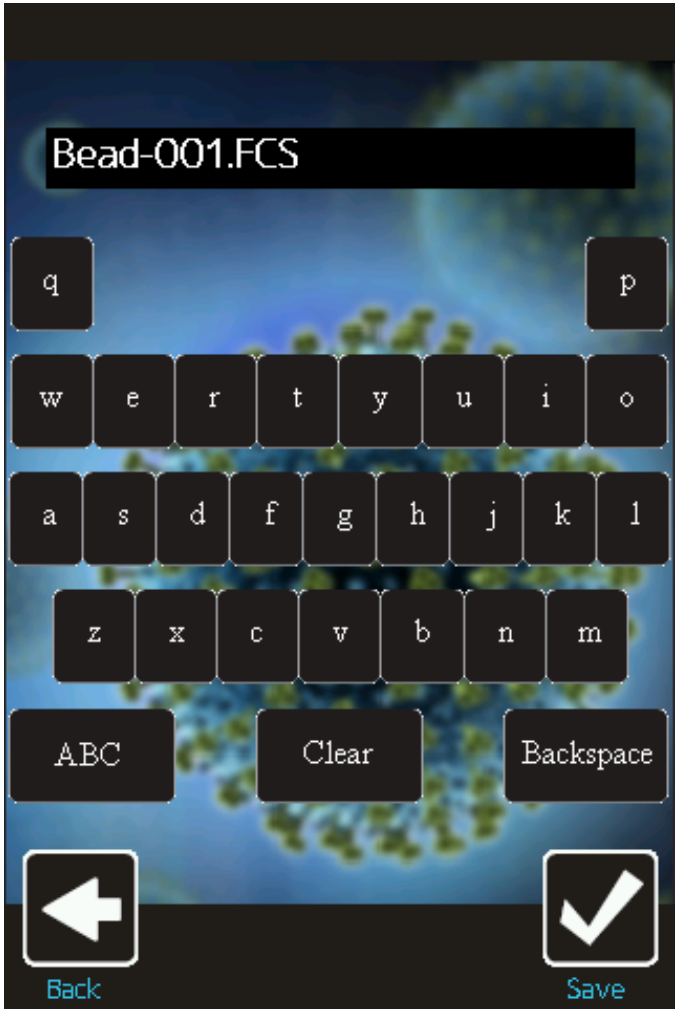
Open Flow Mode

3 different gain levels can be selected



Touch here and the different gain settings will appear
Select either high, medium, default or low
If you have a known low expressing marker like CD19 set gain to high to create more separation

Naming File



- After touching file name (appears in green box) this screen will appear, which allows you to name the file
- .FCS is done automatically, no need to type in the .FCS

Key Takeaways

ORFLO Revolutionizing Life Science

- Be sure to dilute so cells are in optimal concentration range
  - 1,000-1.5M cells/ml for S-Cassettes
  - 1,000 – 500K cells/ml for M-Cassettes
- Cells need to be in size range of our cassettes
  - S-cassettes: 2.8-18um mean diameter
  - M-cassettes: 10-25um mean diameter
- Always run experiments with instrument plugged into wall
- Pipette quickly and don’t worry about the bubble that forms it will be drawn in by our vacuum pump

Moxi Flow Bench Mark Table

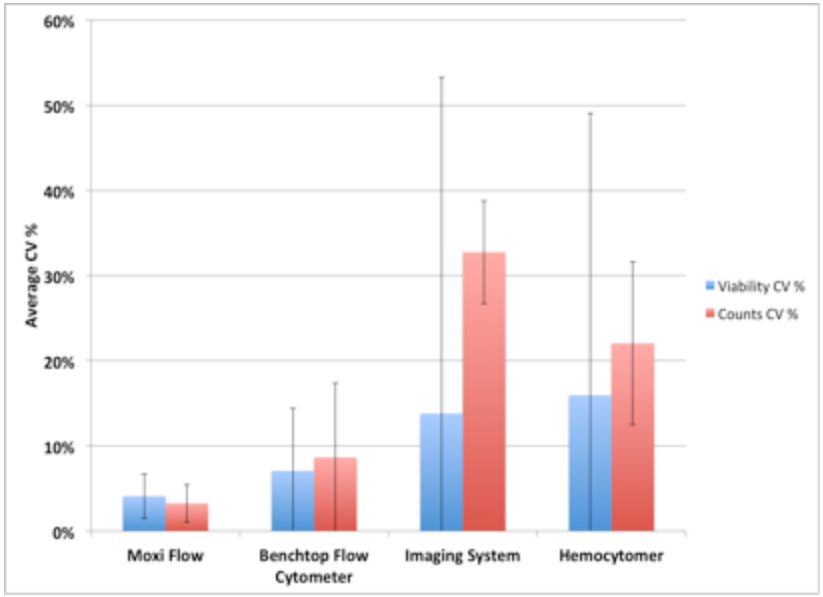


Fig. 4– Average coefficients of variation (CV) percentages for both counts and viability measurements for all systems. Error bars represent one standard deviation.

Moxi Flow Go To Market Approach (RUO)

ORFLO Revolutionizing Life Science

Pitch as Rapid, "Plug & Play" Affordable Protein and Cell Analyzer Targeting 4 Key Category Areas in Cell and Protein Biology

1. **Open Platform Flow Cytometry**

   PE, Sytox Orange, Nile Red, Suncoast Yellow, Ethidium Homodimer
   Single marker, transfection efficiency, purity checks
   Under the hood experimentation, trend & time course

2. **Powerful Immunology Analyzer**

   4 Part Diff. WBC's counts (lympho, mono, granulacyte)
   Single CD marker expression
   Instant direct size confirmation

3. **Multiplex quantitative protein expression analysis**

   "on-demand"
   1-6 plex bead assays

4. **Cell Health & QC**

   Gold Standard Counts, Viability
   Apoptosis, Cell Cycle
   Microbe contamination
   "Quick-Check" Cell Volume, morphology

Target 700K researchers across well defined customer segments

- Cell Biology
- Cancer, Neuro, Metabolism Immunology
- Stem Cell Labs
- Tissue Culture
- PD Groups
- Flow Cores

1. *In most cases lead with "open flow" have customers prepare samples ahead of demo (PE labeled flow ab)*
2. *During demo customer will begin thinking of other ways to use the system*

Bench Top Flow Competitive Landscape

ORFLO Revolutionizing Life Science

Brand	Moxi Flow	Muse	Accuri C6	easyCyte	MACSQuant
MFG	Orflo	EMD Millipore	BD	EMD Millipore	Partec
Price	$9,995	$15,000	$70,000	$50-125K	$80-120K
Features	• 1 Laser (532), 1 PMT (590/40) • Direct size (3D+2D) via coulter principle • Disposable flow cell • 50ul sample • 10 second read time • 2lbs 11 oz (3"x 6" X 5") • Battery and AC powered • <3% CV and <5% error, count & viability	• 1 Laser (532nm – green) • 2 colors (YLW, Red), FSC • Cell Counts via forward scatter • 100-300ul sample • 2-3 minute read time • AC powered	• 2 Laser • 4 colors, FSC, SC • 100-200ul sample • 1-2 minute read time • Peristaltic pump creates noise challenge, but lower price • AC powered	• Up to 2 Laser • Up to 6 colors	• 2 Laser • Up to 8 colors, FSC, SC • 2 minute read time
Positioning	• World's first combination flow cytometer under $10K • Direct viability and count, with combined flow cytometry and coulter accuracy and precision • Single cell resolution • Industry leading ease of use • No cleaning no maintenance • Rapid run and analysis time • The ideal cell assay development, QC, Design Of Experiment and Trend analyzer • In-hood capable, blue tooth printing • iPhone like simplicity and power	• Single cell resolution • Low Cost Instrument • Moderate ease of use • Requires cleaning, flow cell subject to clogging and frequent replacement • Closed system • No multiplexing capabilities for bead based assays • Spend 7X more time maintaining than running (from user manual and manufacturer's recommendations)	• Requires skilled flow cytometrist to use • Sheath fluid waste • Spend 7X more time maintaining than running (from user manual and manufacturer's recommendations)	• Complete solution of reagents, analytic • Flow cell enables small sample, less waste • Requires skilled flow cytometrist to use	• Requires skilled flow cytometrist to use • Sheath fluid waste

Multiplex Competitive Landscape

ORFLO Revolutionizing Life Science

				
Brand	Moxi Flow + OS Plex	Luminex LX200, FM3D	Luminex MagPix	MSD
Price	$13,995	$50-120K	$35K	$100-$150K
Features and Specs	• 1 Laser (532), 1 PMT • Direct size via coulter • Disposable cassette • 50ul sample • 10 second per sample read time • 10-4000 pg/ml dynamic range • 3-6 plex capability	• 2 Laser • 3 colors • Plate based	• Image based system • 3 log dynamic range • 1pg/ml sensitivity	• Functional detection limit (binding assays): ~10^6 labels • Dynamic range: 10^5 • Read time: 70 seconds per plate • Cooled CCD camera • Custom telecentric lens • Integrated bar code readers • Two-dimensional motion control • 3 second per sample
Positioning VS Competition	• World's first combination flow and MPX bead analyzer for under $10K • Direct viability and count, with combined flow cytometry and coulter accuracy and precision • Single cell resolution • Industry leading ease of use • No cleaning no maintenance as the cassette = the flow cell • Rapid read time • The ideal cell assay development, QC instrument • In-hood capable	• Extremely complicated front end • Fickle fluidic management • Requires cleaning maintenance • Steep learning curve • Not at all plug and play	• Extremely complicated front end • Fickle fluidic management • Requires cleaning maintenance • Steep learning curve • Not at all plug and play	• Plex limited at 10 • Fickle fluidic management • Requires cleaning maintenance • Steep learning curve • Not at all plug and play

Product Comparison Heat Map Versus Imaged Based Approaches (Viability and Cell Counting)

ORFLO Revolutionizing Life Science

	Moxi Flow	Nucleo-counter	Vi-Cell, Cedex (vision)	Cellometer Luna, Countess, TC20
Viability	100X separation with PI	Yes, PI based, but vision based approach lacks accuracy	2X separation w/ Trypan Blue Exclusion +20% error (accuracy & precision)	
Instrument Cost	$9,995	$15,000-$25,000	$50,000	$19,000-$50,000 Euro
Event Rate	30-50K (10 sec)	100's-1000 (minutes)	5,000 (minutes)	100's (1 minute)
Open Flow Cytometer Apoptosis, Cell Cycle, Multiplex bead assays	Yes	No		
Count	Gold standard Coulter <3% error	1um resolution on size Can't handle complex samples	Less than 20% accuracy and precision Can't handle complex samples (RBC lysed whole blood, PBMC's)	
Cell Diameter	10nm resolution	1um resolution	1um resolution	No
Cell Volume	Yes		No	
Mean Cell Volume	Yes	2D image only	No	
In Hood Use	Yes		No	
Test Time and sample volume	10 sec 50 ul	30 seconds	1-2 minutes 500ul	1-2 minutes 100 ul
Instrument Cost	$9,995	$14,000-20K	$40,000-$50,000	$5,00-20,000
Running cost (US LP)	$2.14 per test	$2.66/Cartridge plus two reagents	500ul sample, time, $1.14 reagents per sample	Repeats required, time, not reliable Up to $3/test w/ 3 repeats

Traditional Flow Cytometry Drawbacks

ORFLO Revolutionizing Life Science

- Significant cost and time associated with non-value added activities
  - Startup, shutdown, cleaning, decontamination, de-clogging, maintenance, calibration, sheath fluid consumption (as per manufacturers recommendation) and waste disposal
- Overkill capability and complexity
  - For most experiments 1 log separation is all that is needed for an accurate/precise answer
  - More than two colors, multiple laser functionality serves niche need in market place
  - 96 well plate automation is also overkill for the everyday researcher who studies 3-6 samples per week
  - Moxi Flow single color and impedance satisfies large unmet need for rapid, accurate, precise answers to scientific questions, under the hood
- Most bench top service plans exceed the cost of the Moxi Flow!
- Traditional Flow Cytometers are PC based controlled with complicated software
  - Complicated and overkill for majority of applications that the “every-day” researcher is focused on

Moxi Flow Truly is the iPhone of Flow Cytometry

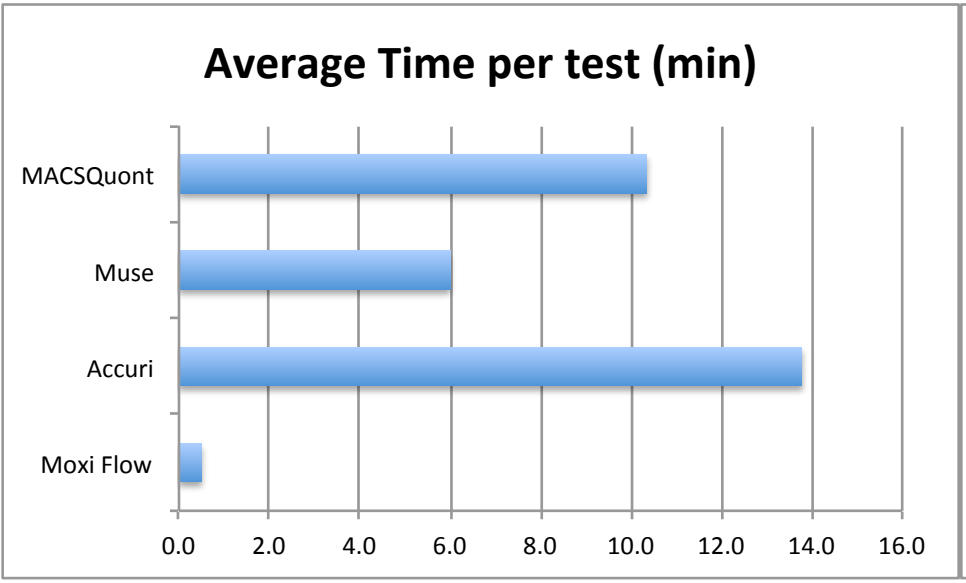
Intuitive, Powerful, Fast, Affordable and Allows Researchers to spend their time on research, not non-value added activities

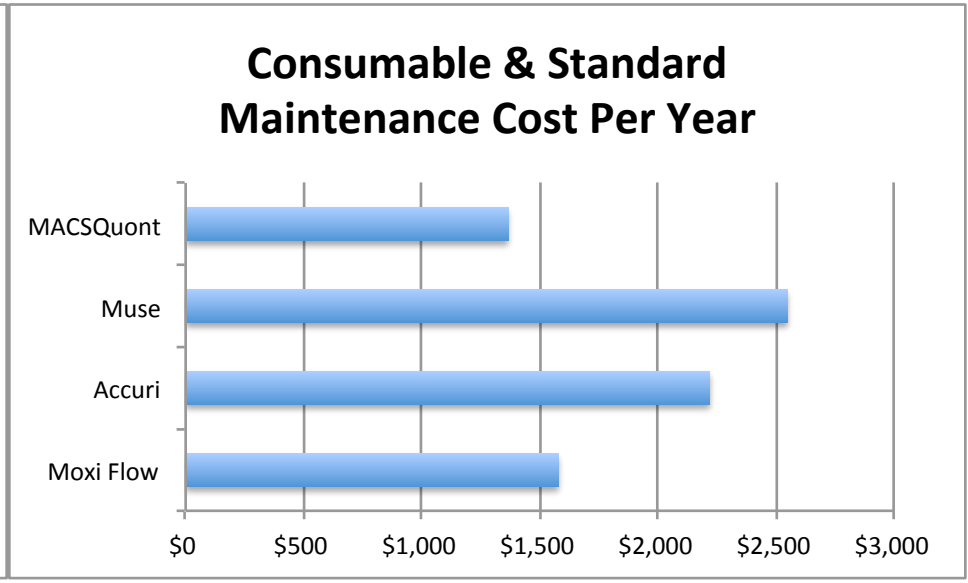
Summary of Non-Value Added Cost and Time Using Traditional Flow Cytometers

ORFLO Revolutionizing Life Science

Utilization Assumptions Per Instrument	
Usage rate (per week)	5
Average tests per day	3
Weeks per year	50
Assumed rate of clogging as a % of total annual tests	5%
Average tests per year (used as basis for each platform)	750
Labor Rate in $'s fully burdened	$40

Total Cost & Time Associated with NON-VALUE Added Activities	Consumable & Standard Maintenance Cost Per Year	Consumable & Standard Maintenance Cost Per Test	Time per year (hours)	Average Time per test (min)	Annual Labor Cost	Per Test Labor Cost	Total Cost Per Test	Total Annual Cost
Moxi Flow	$1,575			**0.5**				
Accuri	$2,217	$3.69	138	13.78	$5,513.33	$9.19	$12.88	$7,730
Muse	$2,545	$4.24	60	6.00	$2,400.00	$221.69	$225.93	$4,945
MACSQuont	$568	$0.95	104	10.35	$4,140	$6.90	$7.85	$4,708





Accuri Hidden Costs and Time Detailed Analysis

ORFLO Revolutionizing Life Science

Ratio of Maintenance Time to Actual Analysis Time = 13.8 Minutes/2 minutes (7X)
Weight: 30 lbs (11" x 14.75" x 16.5")

ACCURI - BD

Recommended by Manufacturer	Source	Frequency (per year)	Time Required MFG Recommended Step (minutes)	Volume of solution used per activity (ml)	Cost per ml	Cost per year (using test per year assumptions)	Cost per test (averaged out over 1 year)	Total Time Per Year (min)	Time Per Test (min)
Cleaning, decontamination, de-clogging & Washing									
Power on sytem flush	Usesr guide	250	10	8	$0.03	$66.00	$0.09	2500	3.3
Shut down decontamination	Usesr guide	250	10	4	$0.18	$175.00	$0.23	2500	3.3
De-clog (assume once/week)	User Manual	50	30	4	$0.03	$1.65	$0.00	1500	2
Wash flow cell after each sample	Usesr guide	750	5	2	$0.03	$49.50	$0.07	3750	5
				Total Wash/Decontamination Costs & Time		***$292.15***	***$0.39***	**10,250**	**13.7**

Maintenance	Source	Frequency per year	Time Required MFG Recommended Step (minutes)	Kit Cost	Cost per Year	Cost per test (averaged out over 1 year)	Total Time Per Year (min)	Time Per Test (min)
Laser Alignment	Industry expert	1			$200.00	$0.27		
2 month maintenance (tubing, filters	User Manual	6	10	$150.00	$900.00	$1.20	60	0.08
1 Year Maintenance	User Manual	1	10	$825.00	$825.00	$1.10	10	0.01
			Total Routine Maintenance Costs		***$1,925.00***	***$2.57***	***70***	***0.09***

Consumables Used During Test	Sheath Fluid Flow Rate (ml/min)	Average Run Time (seconds)	Volume of solution used (ml)	Cost/ml of Bacteriostatic Concentrate	Cost of DI H20 per ml	Cost per Year	Cost per test (averaged out over 1 year)
Sheath fluid used in typical run	6	152	15.2	$0.0055	$0.0009	$72.73	$0.10
Calibration							
Cassette	NA	NA	NA	NA	NA	NA	NA

WASTE Disposal	Liters
Volume of biological waste per year	16.1

	Cost Per Year	Cost Per Test	Time per year (hours)	Average Time per test (min)	Annual Labor Cost	Per Test Labor Cost	Total Cost Per Test	Total Annual Cost
Total Cost & Time Associated with NON-VALUE Added Activities	$2,289.88	$3.05	172	13.76	$6,880	$9.17	$12.23	$9,169.88

Muse Hidden Costs and Time Detailed Analysis

ORFLO Revolutionizing Life Science

Ratio of Maintenance Time to Actual Analysis Time = 6 Minutes/2 minutes (3X)
(Weight = 13.1 lbs, 8.1” x 11.1” x 8.7”)

Muse - EMD Millipore

Recommended by Manufacturer	Source	Frequency (per year)	Time required to perfor step (minutes)	Volume (units or ml)	Cost per unit or ml	Cost per year (using test per year assumptions)	Cost per test (averaged out over 1 year)	Total Time Per Year (min)	Time Per Test (min)
System Check, Cleaning, Decontamination									
Power on system Check (beads, 3X)	Usesr guide (pg 46)	250	5	3	$2.00	$1,500.00	$2.00	1250	1.7
Capillary rinse (Guava ICF)	Usesr guide	250	2	0.50	$0.33	$41.25	$0.06	500	0.7
Complete clean (end of each day)	Usesr guide (pg 59)	250	5	1.00	$0.33	$82.50	$0.11	1250	1.7
Quick clean	User Manual	250	2	0.50	$0.33	$41.25	$0.06	500	0.67
De-clog (assume once/week)	Usesr guide	50	20	1.00	$0.33	$16.50	$0.02	1000	1.33
				Total Wash/Decontamination Costs & Time		***$1,681.50***	***$2.24***	**4,500**	**6.0**

Consumables Used During Test	Replacements per year				Cost	Cost per Year	Cost per test (averaged out over 1 year)
Flow Cell (VOC)	4	152	10.1	$0.0055	$400.00	$1,600	$2.13
Cassette	NA	NA	NA	NA	NA	NA	NA

	Cost Per Year (materials)	Cost Per Test	Time per year (hours)	Average Time per test (min)	Annual Labor Cost	Per Test Labor Cost	Total Cost Per Test	Total Annual Cost
Total Cost & Time Associated with NON-VALUE Added Activities	$3,281.50	$4.38	75	6.00	$3,000.00	$22.90	$27.27	$6,281.50

MACSQuant Hidden Costs and Time Detailed Analysis

ORFLO Revolutionizing Life Science

Ratio of Maintenance Time to Actual Analysis Time = 10.4 Minutes/2 minutes (5X)
Weight: 100 lbs (23.6" x 13.8" x 15.7")

MACSQuant - Miltenyi

Recommended by Manufacturer	Source	Frequency (per year)	Time Required MFG Recommended Step (minutes)	Volume of solution used per activity (ml)	Cost per ml	Cost per year (using test per year assumptions)	Cost per test (averaged out over 1 year)	Total Time Per Year (min)	Time Per Test (min)
Cleaning, decontamination, de-clogging, calibration & Washing									
Power on sytem flush	Tech Service Chat	250	7	6.6	$0.03	$54.45	$0.07	1750	2.3
Shut down decontamination	Usesr guide	250	7	4	$0.00	$4.44	$0.01	1750	2.3
De-clog (assume once/week)	Industry Expert	5	30	4	$0.03	$0.17	$0.00	150	0.2
Calibration (automated)	User Manual	250	20	0.1	$38.00	$950.00	$1.27		
Wash, clean and backflush	Tech Service Chat	250	16	6.6	$0.02	$24.75	$0.03	4000	5.3
				Total Wash/Decontamination Costs & Time		***$1,033.81***	***$1.38***	**7,650**	**10.2**

Maintenance	Source	Frequency per year	Time Required MFG Recommended Step (minutes)	Unit Cost	Cost per Year	Cost per test (averaged out over 1 year)	Total Time Per Year (min)	Time Per Test (min)
Exchanging Hydrophobic Air Filters	User Manual	1	10	$30.00	$30.00	$0.04	**10**	0.01
Laser re-alignment	Tech Service Chat	1	60	$150.00	$300.00	$0.40	60	0.08
			Total Routine Maintenance Costs		***$330.00***	***$0.44***	***60***	***0.08***

Consumables Used During Test	Sheath Fluid Flow Rate (ml/min)	Average Run Time (seconds)	Volume of solution used (ml)	Cost per ml	Cost per ml	Cost per Year	Cost per test (averaged out over 1 year)
Sheath fluid used in typical run (tech service chat)			6.6	$0.0039	$0.0009	$23.71	$0.03
Cassette	NA	NA	NA	NA	NA	NA	NA

WASTE Disposal	Liters
Volume of biological waste per year	9.3

	Cost Per Year	Cost Per Test	Time per year (hours)	Average Time per test (min)	Annual Labor Cost	Per Test Labor Cost	Total Cost Per Test	Total Annual Cost
Total Cost & Time Associated with NON-VALUE Added Activities	$1,387.51	$1.85	129	10.28	$5,140	$6.85	$8.70	$6,527.51

Flow Performance Comparison

ORFLO Revolutionizing Life Science

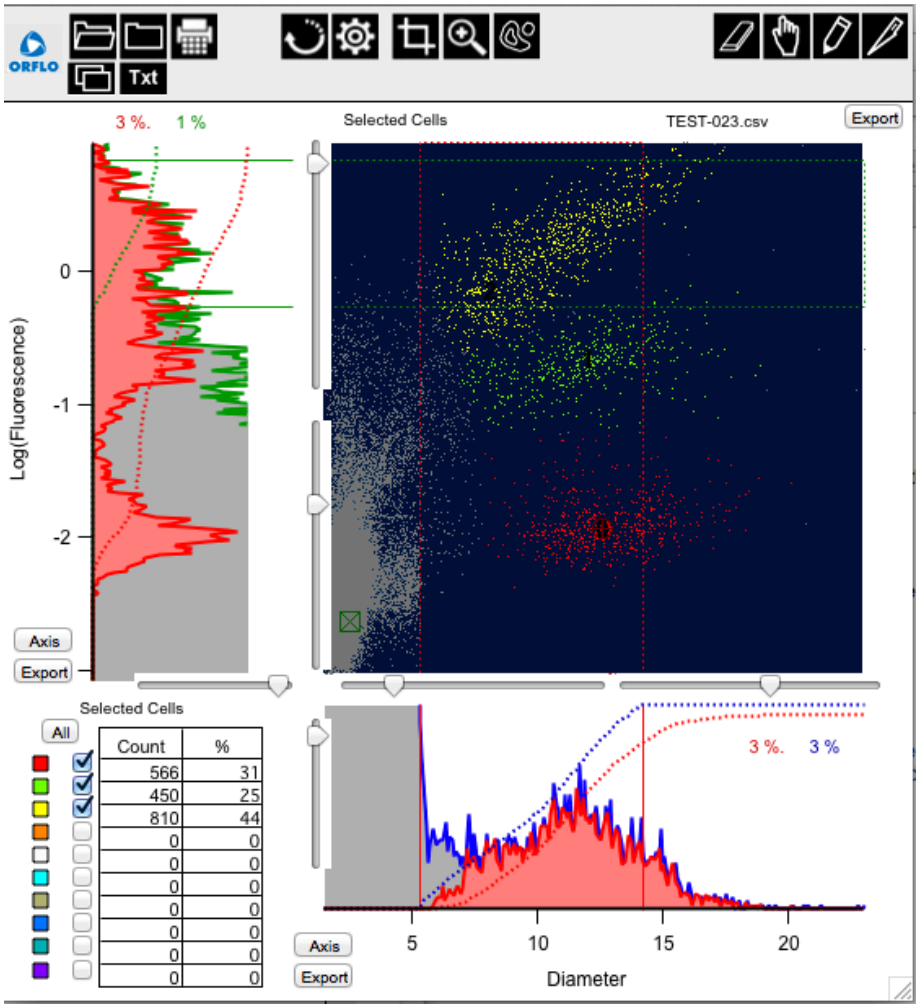
Parameter	*Moxi Flow*	*Accuri*	*Muse*	*MacsQuant*
Sample size (ul), (competitors sample sizes are determined from recommended User Manuals by suppliers)	**27**	**100**	**100**	**100**
Flow rate (ul's/sec)	**2.73**	**0.66**	**0.36**	**0.83**
Average Cell Concentration per test (cells/ul)	**1,000**	**500**	**250**	**500**
Average Test time (seconds)	**10**	**152**	**278**	**120**
Cells analyzed per second	**2,730**	**330**	**90**	**417**
Average number of cells analyzed per test	**27,300**	**50,000**	**25,000**	**50,000**

Moxi Flow Outperforms Instruments that are 4-10X It's cost

Vestigo – Flow Analysis Software

- Intuitive UI for a broader cytometry user base
- Flow Cytometry Standard (FCS) compatibility
- Automated 2D Gaussian Curve-Fitting
  - Maximum likelihood estimation
  - Removes subjectivity of gating analysis
  - Precise population core identification
  - Fully automated or user-defined regions
- Identifies potential “hidden” populations
- Easily groups identified regions

- Demo using web-ex



Moxi Flow, Single Color Flow Standard CD Markers

Sample Set Overview (test date: 20130913)
All prep performed by Elena Wang, Biolegend Custom Specialists

ORFLO Revolutionizing Life Science

- Sample Cell Type: Peripheral Human Blood
- Sample Preparation:
  - RBC Lyse http://www.biolegend.com/rbc-lysis-buffer-10x-1498.html
  - PE anti-human antibody stain with Biolegend reagent
    - Sample 1 (test 18): Stained with BioLegend PE anti-human CD3 Antibody (100ul/ 100ul of whole blood)
    - Sample 2 (test 19): Stained with BioLegend PE anti-human CD4 Antibody (100ul/ 100ul of whole blood)
    - Sample 3 (test 20): Stained with BioLegend PE anti-human CD8a Antibody (100ul/ 100ul of whole blood)
    - Sample 4 (test 22): Stained with BioLegend PE anti-human CD45 Antibody (100ul/ 100ul of whole blood)
  - All samples were then tested on the LSR pre-dilution
  - After LSR testing a 10 to 1 dilution was performed for the Moxi Flow experiments (less than 2 minutes)
  - 5 Moxi Flow runs were performed in 1-2 minutes

Whole Blood Reference Table

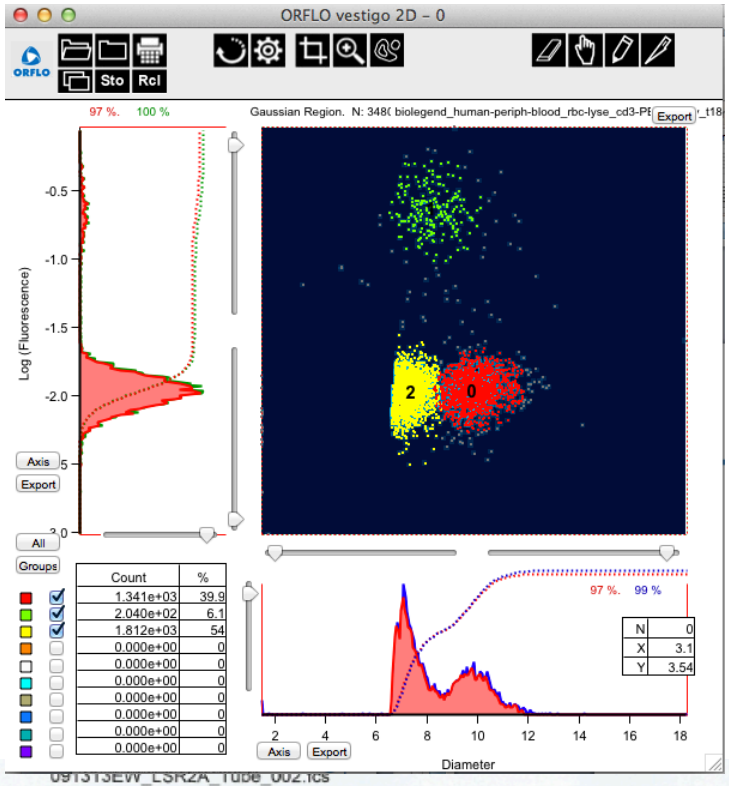
ORFLO Revolutionizing Life Science

Component	Count/ml	Relative %	Diameter (um)	Class
Lymphocyte	1.7-3.5M	25-35%	7-8	aGranulocyte
Monocyte	200-600K	4-6%	14-17	aGranulocyte
Neutrophil	2.5-7M	50-70%	10-12	Granulocyte
Eosinophil	100-300K	1-3%	10-12	Granulocyte
Basophil	40-100K	0.4-1%	12-15	Granulocyte
Total WBC	4.5-10M			

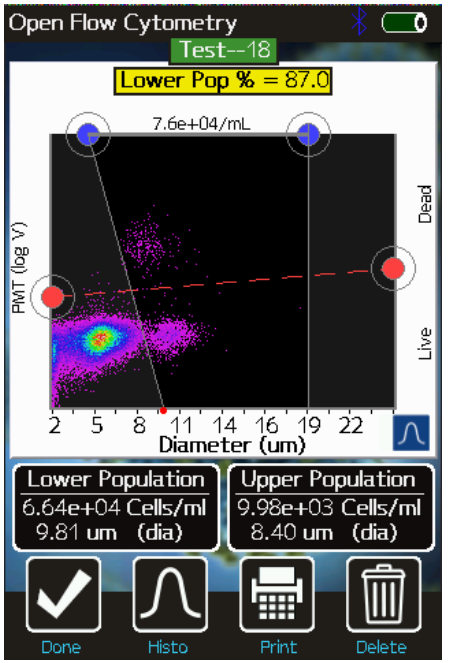
Component	Count/ml	Relative %	Diameter (um)	Class
RBC	5B		5-6	RBC
Platelets	150-450K		2-3	

PE CD3 Anti Human Stained Peripheral Blood Sample

ORFLO Revolutionizing Life Science



Cropped to include only WBC's
Granulocytes (Pop 0): 40% of total WBC
Lymphocytes (Pop 2): 54% of total
CD3+ (green): 6.1% of total WBC

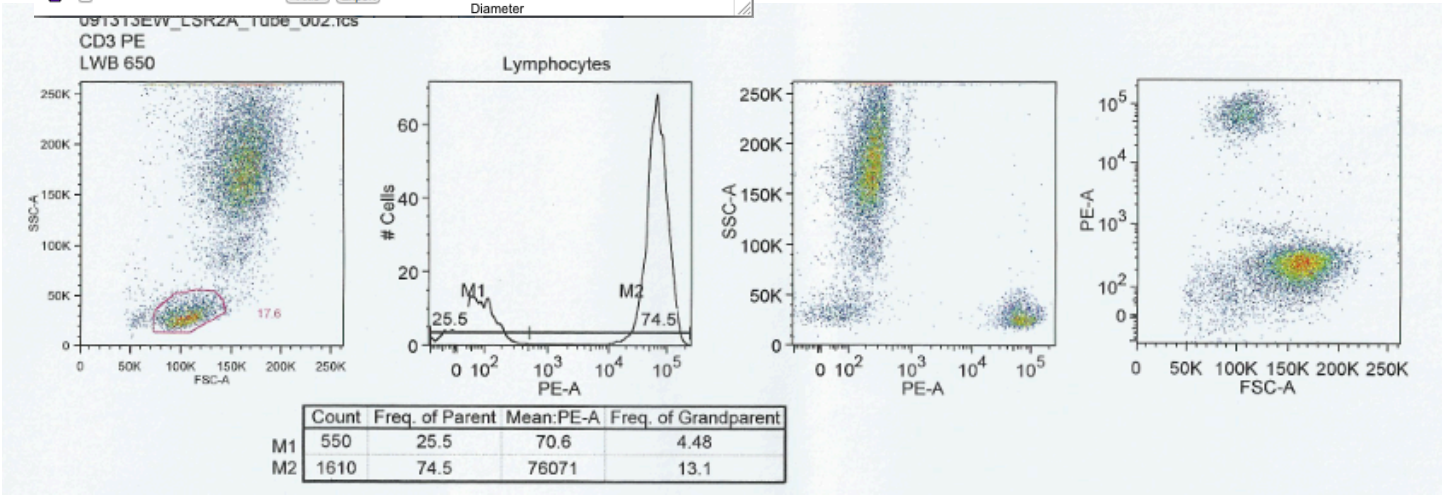


Comments

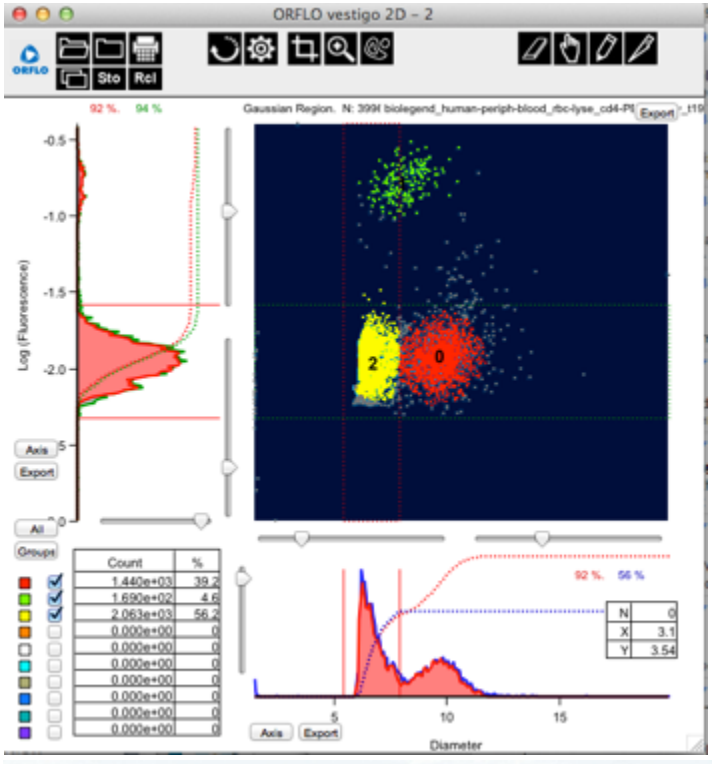
- Dynamic range
  - 1.3 log (MF) vs 3.0 (LSR)

Moxi Flow provides very clear and sufficient separation of the primary stained CD4+ lymphocytes

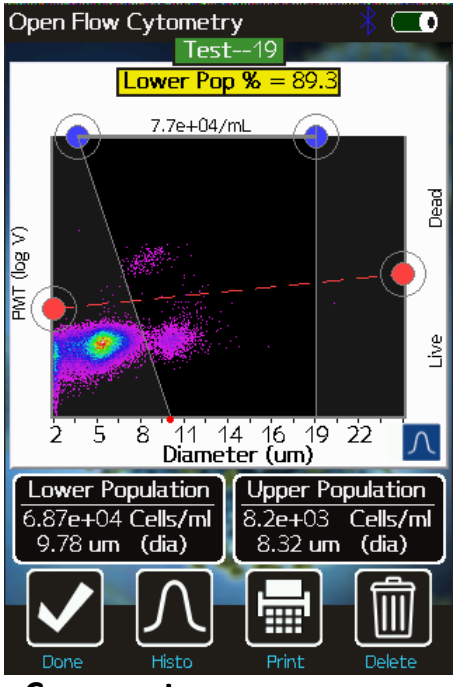
Also has the advantage of impedance channel and providing direct and instant confirmation



PE CD4 Anti Human Stained Peripheral Blood Sample

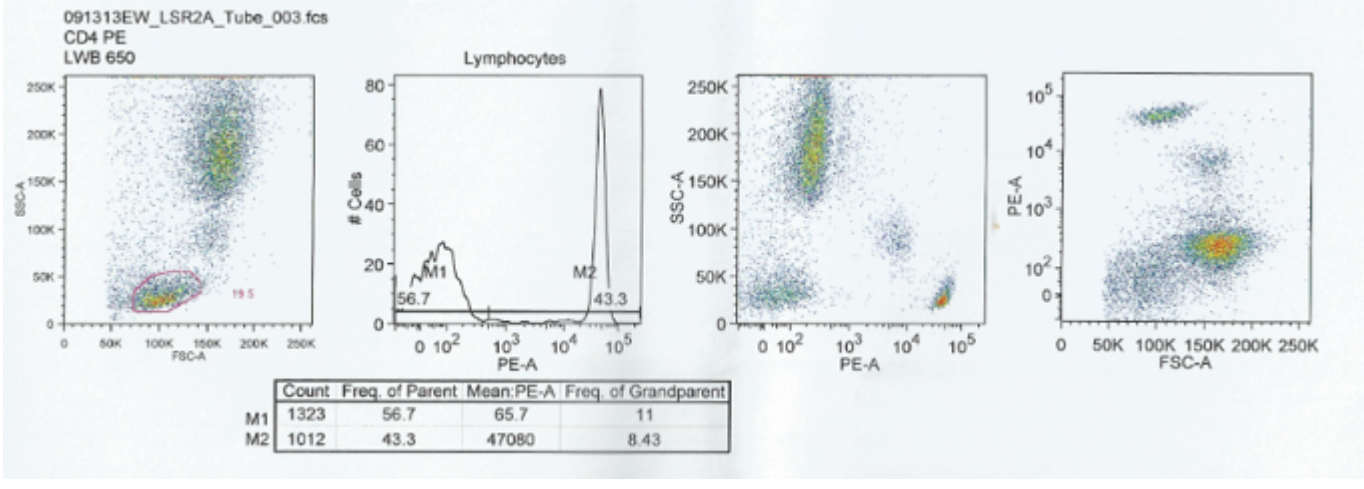


Cropped to include only WBC's
Granulocytes (Pop 0): 39% of total WBC
Lymphocytes (Pop 2): 56% of total
CD4+ (green): 4.5% of total WBC

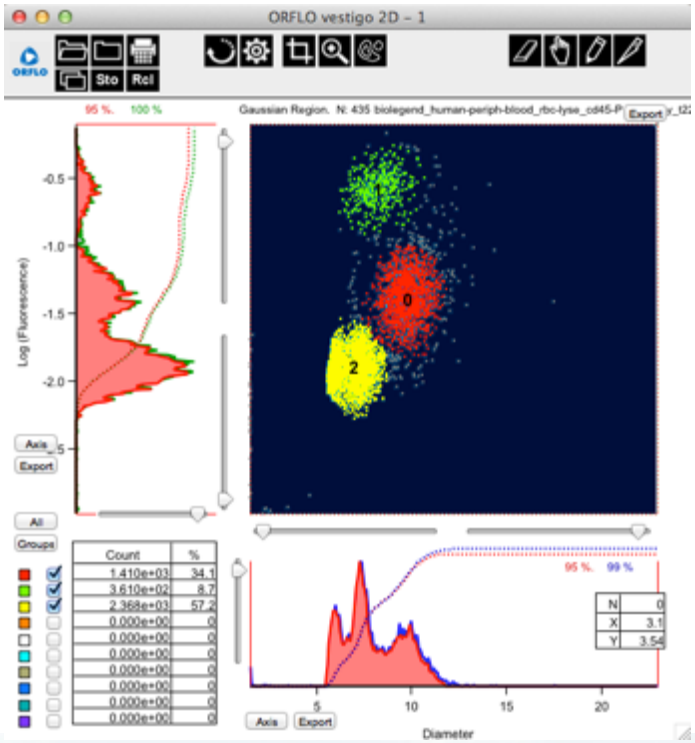


Comments

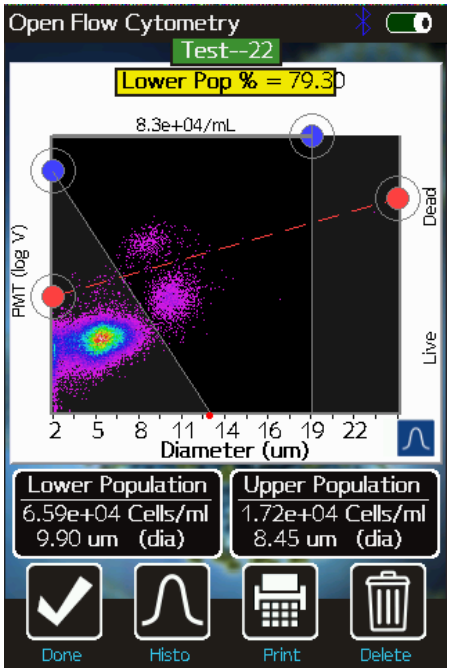
- Dynamic range
  - 1.8 log (MF) vs 2.0 (LSR)
- Moxi Flow provides very clear and sufficient separation of the primary stained CD4+ lymphocytes
- Also has the advantage of impedance channel and providing direct and instant confirmation

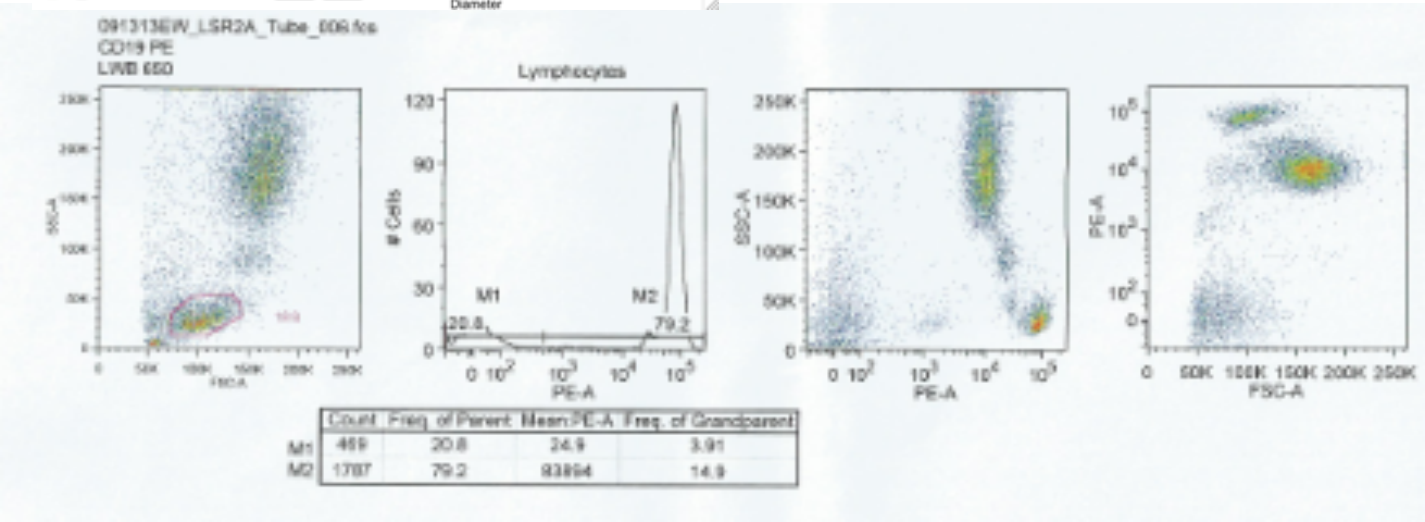


PE CD45 Anti Human Stained Peripheral Blood Sample



Cropped to include only WBC's
Granulocytes (Pop 0): 34% of total WBC
Lymphocytes (Pop 2): 57% of total
CD45+ (green): 8.7% of total WBC

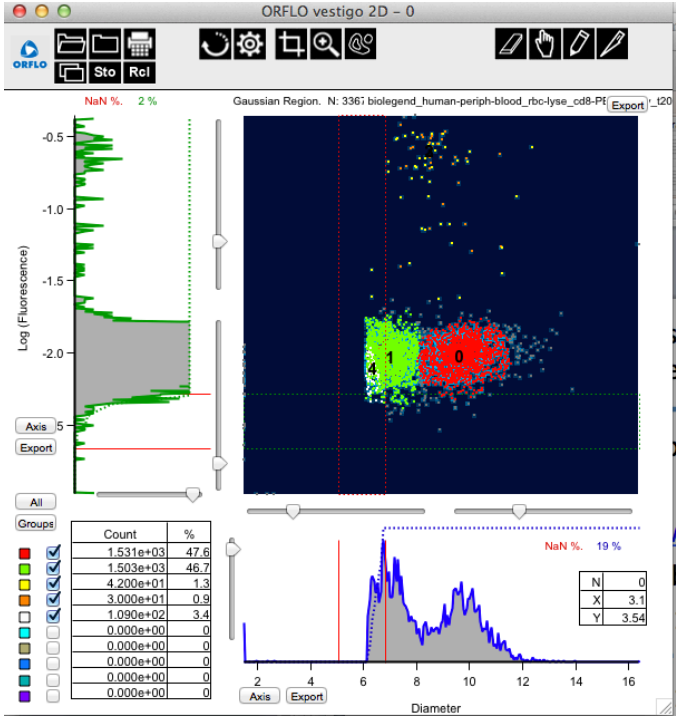




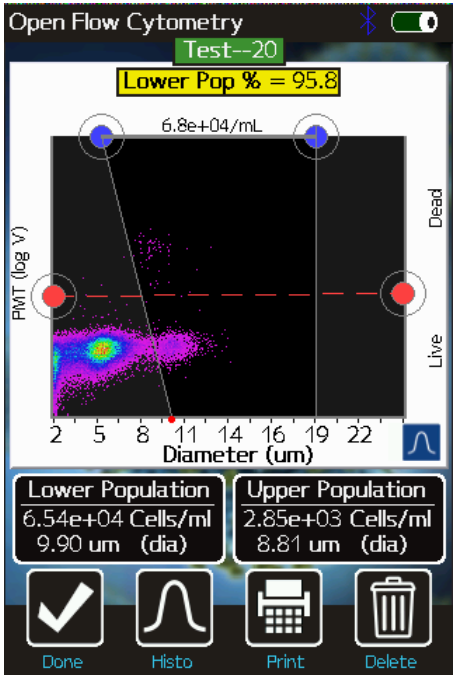
Comments

- Dynamic range
  - 1.5 log (MF) vs 3 (LSR) (cd45+ lympho's)
  - 0.5 log (MF) vs 2 (LSR) CD45+ (granulocytes)
- Moxi Flow provides very clear and sufficient separation of the primary stained CD4+ lymphocytes
- Also has the advantage of impedance channel and providing direct and instant confirmation

PE CD8 Anti Human Stained Peripheral Blood Sample

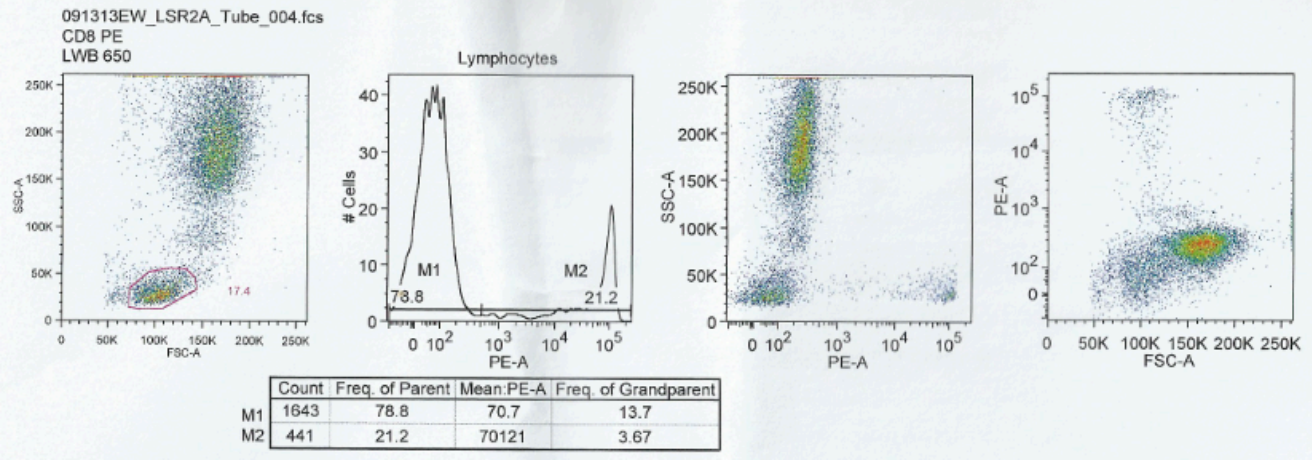


Cropped to include only WBC's
Granulocytes (Pop 0): 47% of total WBC
Lymphocytes (Pop 1): 47% of total
Monocytes (grey): 1%
CD8+ (yellow): 1.2% of total WBC

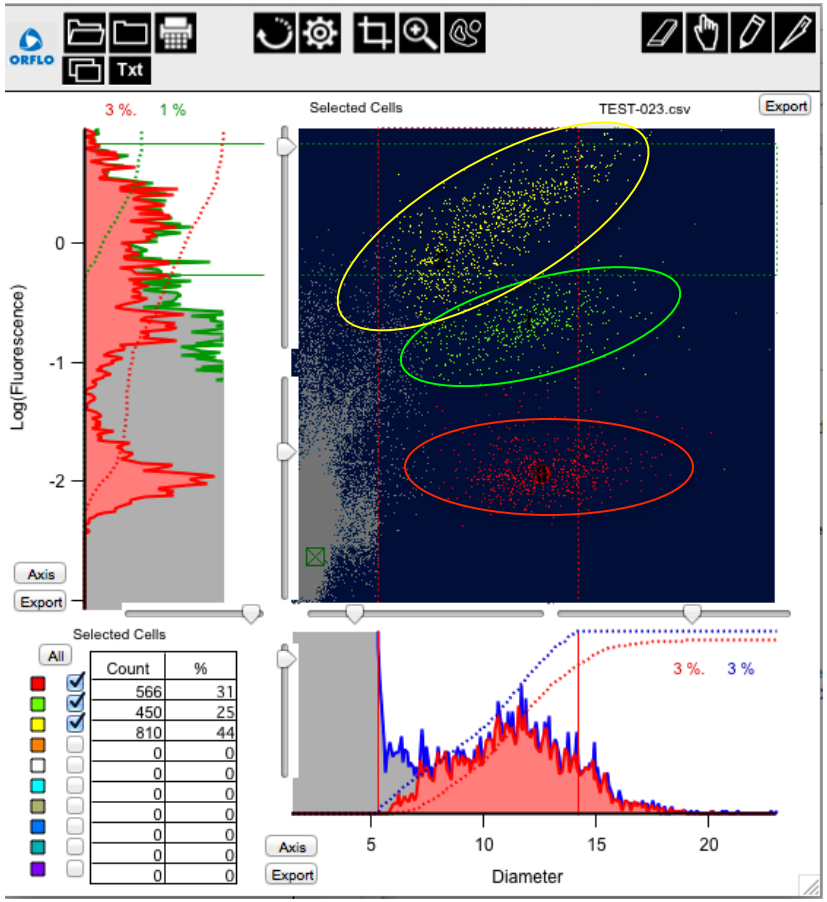


Comments

- Dynamic range
  - 1.5 log (MF) vs 3 (LSR) (cd8+ lympho's)
- Moxi Flow provides very clear and sufficient separation of the primary stained CD4+ lymphocytes
- Also has the advantage of impedance channel and providing direct and instant confirmation



Viability (Sytox Orange) plus AnnexinV Simultaneously



Comments

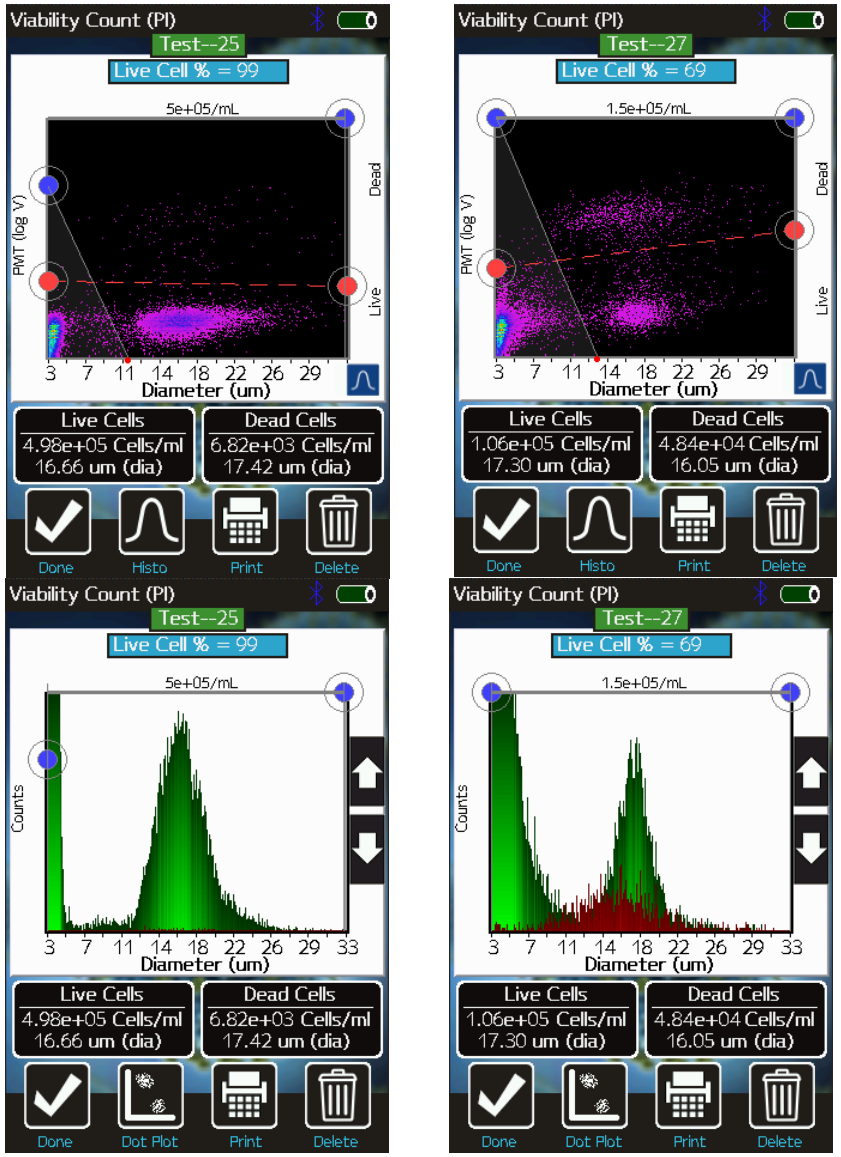
- Clear separation of populations
  - Yellow (Sytox Orange +): Dead
  - Green (AnnexinV +): Apoptotic
  - Red: Live

Examples of Different Applications

Moxi Flow Viability for More Typical Cell Necrosis Approach

- Typical, healthy Suspension CHO culture
- Suspension CHO culture after over-growth of cells (Test 27)
- Clear separation and clustering of dead cells (tight fluorescence distribution and ~1.8 Log (>60x) separation in live vs. dead cells)
- Scatter plots are typical (compared to CHO H_2O_2-killed cell data shown previously) based on viability testing at Orflo
- Moxi Flow provides a unique insight to size distribution through it's direct sizing capability, with 20nm resolution (volume & diameter)

Viability decrease →



Viability decrease →

Sample Set Overview (test date: 20130920)
All prep performed by customer, PE CD4 Experiment

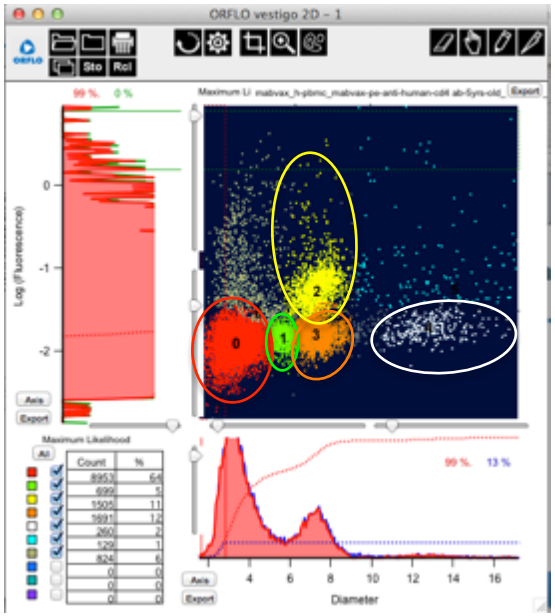
ORFLO Revolutionizing Life Science

- Sample Cell Type: Peripheral Human Blood Mono-nuclear Cytes
- Sample Preparation:
  - Ficoll Paque Centrifugation
  - 4 days of suspension culture in Mabvax Media conditions (note antibody was 4-5 years old)
    - Sample 1 (test 32): Stained with Mabvax PE anti-human CD4 Antibody
    - Sample 2 (test 33): Stained with Mabvax PE anti-human CD4 Antibody
    - Sample 3 (test 34): Stained with Mabvax PE anti-human CD4 Antibody
    - Sample 4 (test 35): Stained with Mabvax PE anti-human CD4 Antibody
    - Sample 5 (test 36): Stained with Mabvax PE anti-human CD4 Antibody
    - Sample 6 (test 37): Stained with Mabvax PE anti-human CD4 Antibody
  - Note Moxi Flow system was running off of battery power
  - All samples were then tested on the LSR pre-dilution
  - After LSR testing a 10 to 1 dilution was performed for the Moxi Flow experiments (less than 2 minutes)
  - 5 Moxi Flow runs were performed in 1-2 minutes

PE CD4 Anti Human PBMC Sample1 (Test 32)

ORFLO Revolutionizing Life Science

Snapshot of Entire Sample

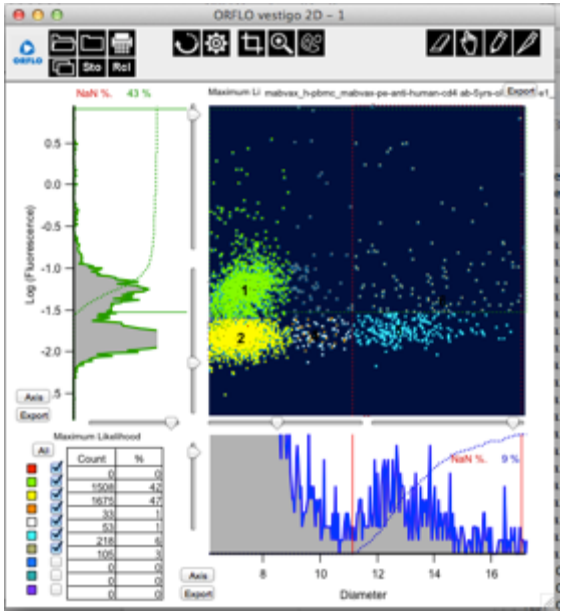


Sample Snapshot	Count	%
Debris (red)	8,953	64%
Residual RBC's (green)	699	5%
CD4- Lymphocytes (orange)	1691	12%
CD4+ Lymphocytes (yellow)	1505	11%
CD4- monocytes (white)	260	2%
CD4+ monocytes (blue)	129	1%
Other debris (grey)	824	6%

Comments

- Dynamic range
  - 0.5 log, some error introduced due to minimal overlap, but estimate this is less than 2-3% error contribution
  - Sufficient separation to quantify CD4 + lymphocytes (green)
- Impedance channel observations
  - Clear direct sizing and counts of CD4+&- lymphocytes, as well as the monocytes (blue)
  - Impedance channel reveals small residual granulocyte population, despite Ficoll-Paque Prep
  - Enables amazing sensitivity on count, down to as few as 33

PBMC Analysis

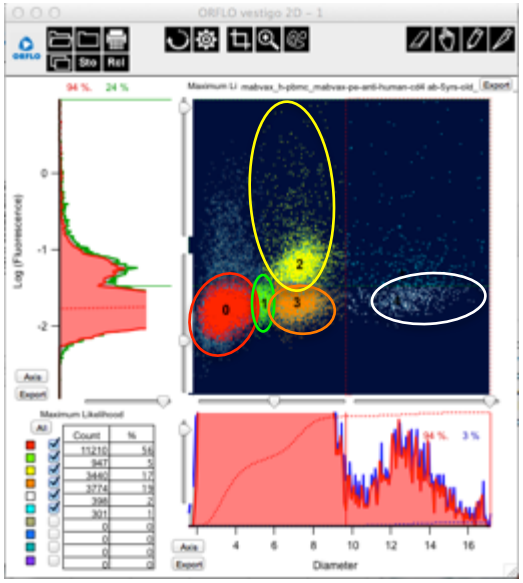


Cell	Count	%
CD4+ Lymphocytes (green)	1,508	42%
CD4- Lymphocytes (yellow)	1,675	47%
CD4- Monoctyes (blue)	218	6%
CD4+ Monocytes (orange)	33	1%
CD4- Granulocytes	105	3%
CD4+ Granulocytes		

PE CD4 Anti Human PBMC Sample1 (Test 33)

ORFLO Revolutionizing Life Science

Snapshot of Entire Sample

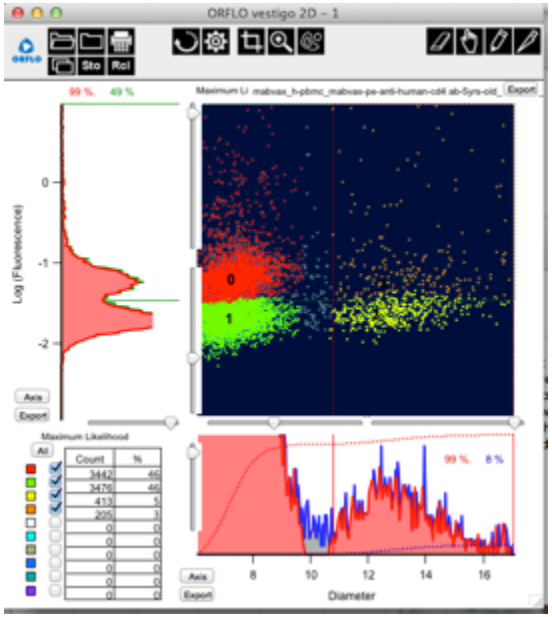


Sample Snapshot	Count	%
Debris (red)	11,210	56%
Residual RBC's (green)	947	5%
CD4- Lymphocytes (orange)	3,440	17%
CD4+ Lymphocytes (yellow)	3,774	19%
CD4- monocytes (white)	398	2%
CD4+ monocytes (blue)	301	1%
Other debris (grey)	824	6%

Comments

- Dynamic range
  - 0.5 log, some error introduced due to minimal overlap, but estimate this is less than 2-3% error contribution
  - Sufficient separation to quantify CD4 + lymphocytes (green)
- Impedance channel observations
  - Clear direct sizing and counts of CD4+&- lymphocytes, as well as the monocytes (blue)
  - Impedance channel reveals small residual granulocyte population, despite Ficoll-Paque Prep
  - Enables amazing sensitivity on count, down to as few as 33

PBMC Analysis

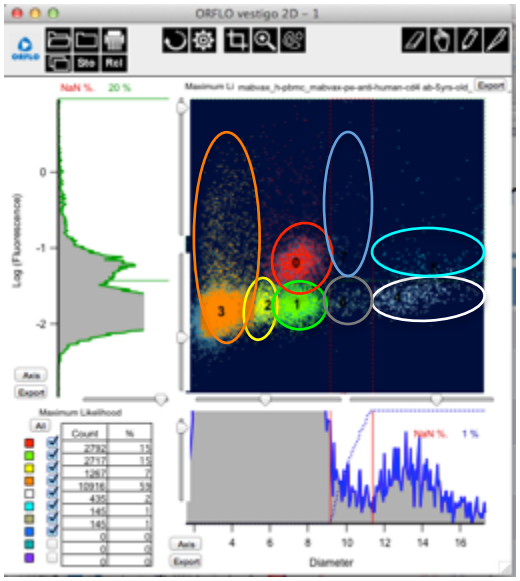


Cell	Count	%
CD4+ Lymphocytes (green)	1,508	42%
CD4- Lymphocytes (yellow)	1,675	47%
CD4- Monoctyes (blue)	218	6%
CD4+ Monocytes (orange)	33	1%
CD4- Granulocytes	105	3%
CD4+ Granulocytes		

PE CD4 Anti Human PBMC Sample1 (Test 34)

ORFLO Revolutionizing Life Science

Snapshot of Entire Sample

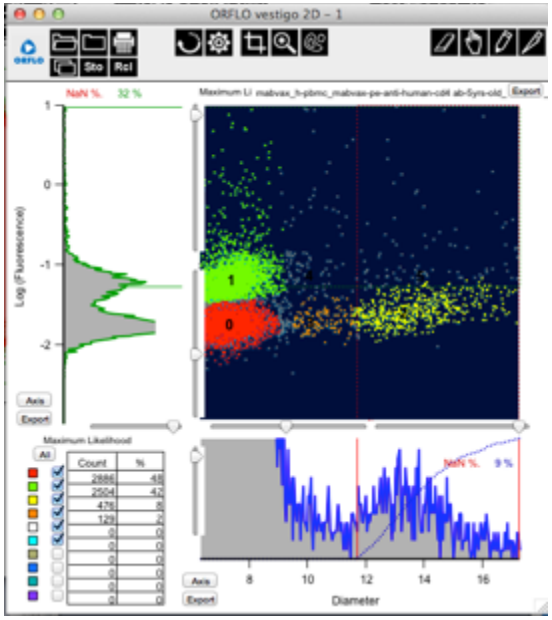


Sample Snapshot	Count	%
Debris (orange)	10,916	59%
Residual RBC's (yellow)	1,267	7%
CD4- Lymphocytes (green)	2717	15%
CD4+ Lymphocytes (red)	2792	15%
CD4- monocytes (white)	435	2%
CD4+ monocytes (blue)	145	1%
CD4- Residual Granulocytes (grey)	145	1%

Comments

- Dynamic range
  - 0.5 log, some error introduced due to minimal overlap, but estimate this is less than 2-3% error contribution
  - Sufficient separation to quantify CD4 + lymphocytes (red) versus CD4- lymphocytes
- Impedance channel observations
  - Clear direct sizing and counts of CD4+&- lymphocytes, as well as the monocytes (blue)
  - Impedance channel reveals small residual granulocyte population, despite Ficoll-Paque Prep
  - Enables amazing sensitivity on count, down to as few as 129 events

PBMC Analysis

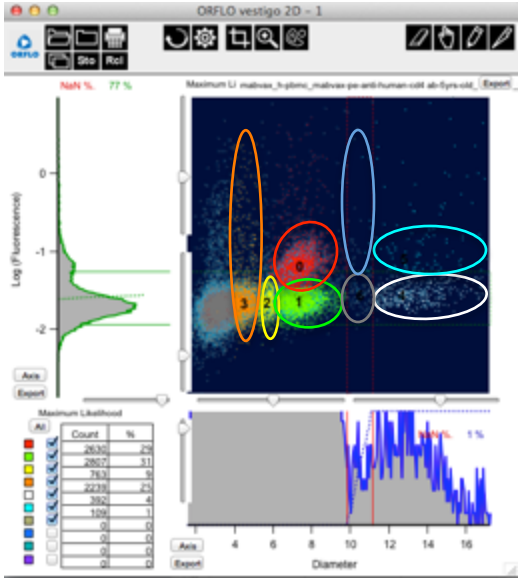


Cell	Count	%
CD4+ Lymphocytes (green)	2,886	48%
CD4- Lymphocytes (red)	2,504	42%
CD4- Monoctyes (yellow)	476	6%
CD4+ Monocytes (grey)	NA	<1%
CD4- Granulocytes (orange)	129	3%
CD4+ Granulocytes (grey)	NA	<1%

PE CD4 Anti Human PBMC Sample1 (Test 35)

ORFLO Revolutionizing Life Science

Snapshot of Entire Sample

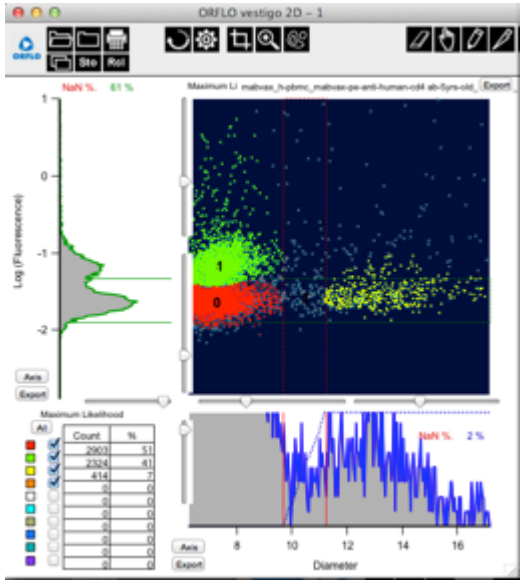


Sample Snapshot	Count	%
Debris (orange)	2,239	25
Residual RBC's (yellow)	763	9
CD4- Lymphocytes (green)	2,807	31
CD4+ Lymphocytes (red)	2,630	29
CD4- monocytes (white)	392	4
CD4+ monocytes (blue)	109	1
CD4- Residual Granulocytes (grey)	NA	<1%

Comments

- Dynamic range
  - 0.5 log, some error introduced due to minimal overlap, but estimate this is less than 2-3% error contribution
  - Sufficient separation to quantify CD4 + lymphocytes (red) versus CD4- lymphocytes
- Impedance channel observations
  - Clear direct sizing and counts of CD4+&- lymphocytes, as well as the monocytes (blue)
  - Impedance channel reveals small residual granulocyte population, despite Ficoll-Paque Prep
  - Enables amazing sensitivity on count, down to as few as 129 event
- Debris region overlaps with system noise

PBMC Analysis

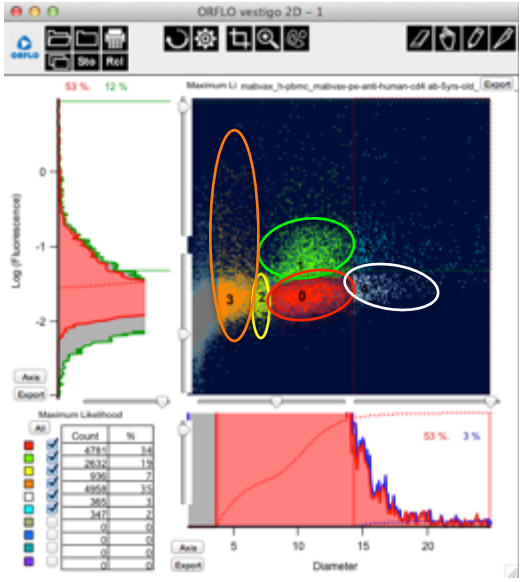


Cell	Count	%
CD4+ Lymphocytes (green)	2,324	41
CD4- Lymphocytes (red)	2,903	51
CD4- Monoctyes (yellow)	414	7
CD4+ Monocytes (grey)	NA	
CD4- Granulocytes (orange)	NA	
CD4+ Granulocytes (grey)		

PE CD4 Anti Human PBMC Sample1 (Test 36, must be the treated sample)

ORFLO Revolutionizing Life Science

Snapshot of Entire Sample

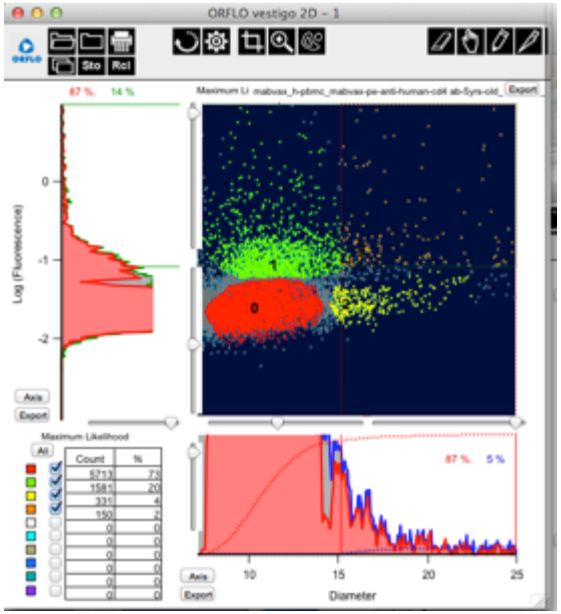


Sample Snapshot	Count	%
Debris (orange)	4,958	35%
Residual RBC's (yellow)	936	7%
CD4- Lymphocytes (red)	4,781	34%
CD4+ Lymphocytes (green)	2,632	19%
CD4- monocytes (white)	365	3%
CD4+ monocytes (blue)	347	2%
CD4- Residual Granulocytes (grey)	NA	

Comments

- Dynamic range
  - 0.5 log, some error introduced due to minimal overlap, but estimate this is less than 2-3% error contribution
  - Sufficient separation to quantify CD4 + lymphocytes (red) versus CD4- lymphocytes
- Impedance channel observations
  - Clear direct sizing and counts of CD4+&- lymphocytes, as well as the monocytes (blue)
  - ***There appears to be a significant morphology change in the PBMC's, significant swelling (30% or more)***
  - Enables amazing sensitivity on count, down to as few as 129 event

PBMC Analysis

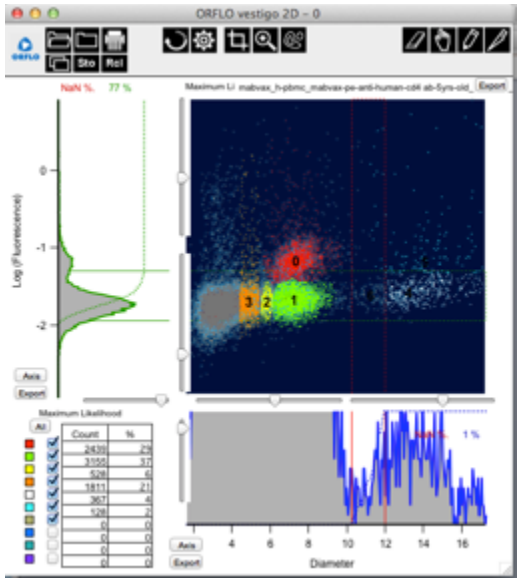


Cell	Count	%
CD4+ Lymphocytes (green)	1,581	20%
CD4- Lymphocytes (red)	5,713	73%
CD4- Monoctyes (yellow)	331	4%
CD4+ Monocytes (grey)	150	2%
CD4- Granulocytes (orange)	NA	
CD4+ Granulocytes (grey)	NA	

PE CD4 Anti Human PBMC Sample1 (Test 37)

ORFLO Revolutionizing Life Science

Snapshot of Entire Sample

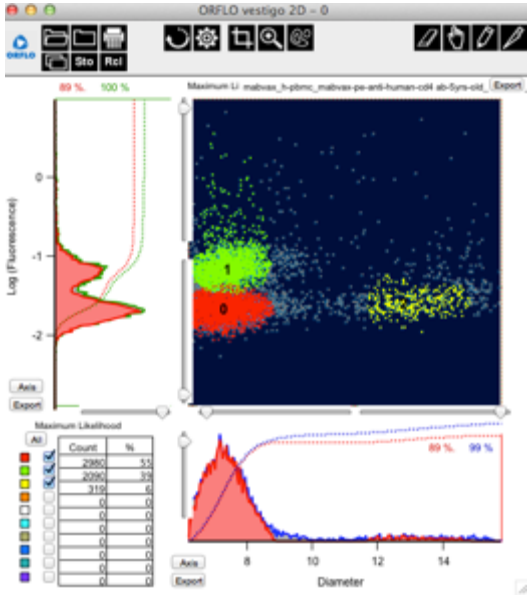


Sample Snapshot	Count	%
Debris (orange)	1,811	21%
Residual RBC's (yellow)	528	6%
CD4- Lymphocytes (green)	3,155	37%
CD4+ Lymphocytes (red)	2,439	29%
CD4- monocytes (white)	367	4%
CD4+ monocytes (blue)	128	2%
CD4- Residual Granulocytes (grey)	NA	

Comments

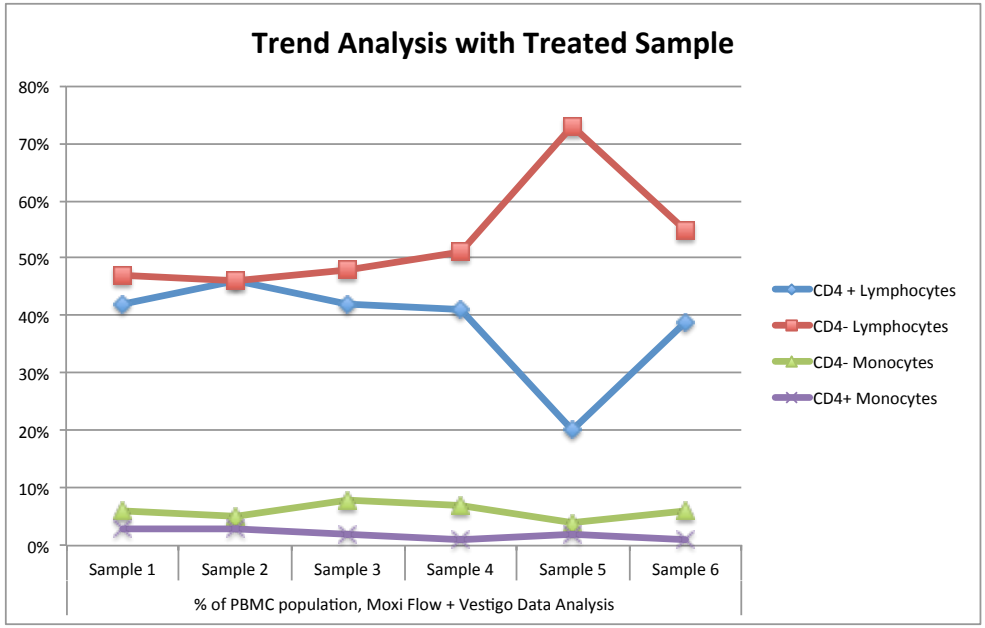
- Dynamic range
  - 0.5 log, some error introduced due to minimal overlap, but estimate this is less than 2-3% error contribution
  - Sufficient separation to quantify CD4 + lymphocytes (red) versus CD4- lymphocytes
- Impedance channel observations
  - Clear direct sizing and counts of CD4+&- lymphocytes, as well as the monocytes (blue)
  - Enables amazing sensitivity on count, down to as few as 129 event
- Debris region overlaps with system noise

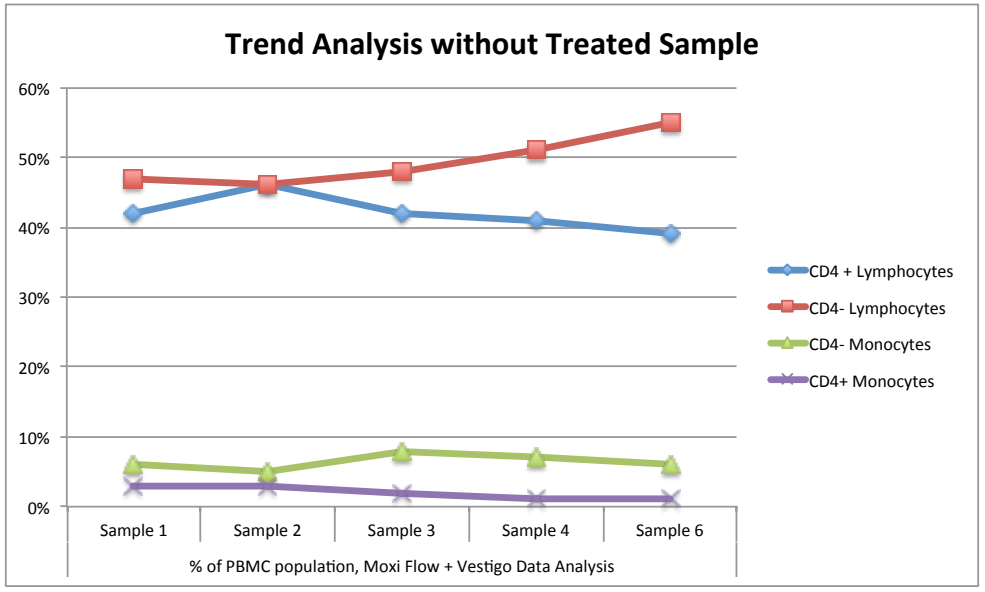
PBMC Analysis



Cell	Count	%
CD4+ Lymphocytes (green)	2,090	39%
CD4- Lymphocytes (red)	2,980	55%
CD4- Monoctyes (yellow)	319	6%
CD4+ Monocytes (grey)		
CD4- Granulocytes (orange)		
CD4+ Granulocytes (grey)		

Summary and Conclusions





	% of PBMC population, Moxi Flow + Vestigo Data Analysis					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
CD4 + Lymphocytes	42%	46%	42%	41%	20%	39%
CD4- Lymphocytes	47%	46%	48%	51%	73%	55%
CD4- Monocytes	6%	5%	8%	7%	4%	6%
CD4+ Monocytes	3%	3%	2%	1%	2%	1%
	Counts of PBMC population, Moxi Flow + Vestigo Data Analysis					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
CD4 + Lymphocytes	1,508	3,442	2,504	2,324	1,581	2,090
CD4- Lymphocytes	1,675	3,476	2,886	2,903	5,713	2,980
CD4- Monocytes	218	413	476	414	331	319
CD4+ Monocytes	105	205	129	50	150	50

Comments

- Moxi Flow enables immediate insight into the quantative levels of expression of CD4 within the PBMC population
- Moxi Flow enables a never seen before direct morphology view in combination with the CD marker expression of PBMC population
- Moxi Flow also enables a view into the level of debris and other residual blood components after Ficoll seperation and 4 days of suspension culture
- Sample 5 was the treated sample that resulted in significant morphology changes of the PBMC's (+ 30% swelling) along with far less CD4 expression
- There is an observable trend of increasing CD4- lymphocytes and decreasing CD4+ lymphocytes in the untreated sample set

ORFLO Revolutionizing Life Science

CustomerX Primary PE CD34 Flow Ab & Viability Evaluation On Moxi Flow

By Don O'Neil

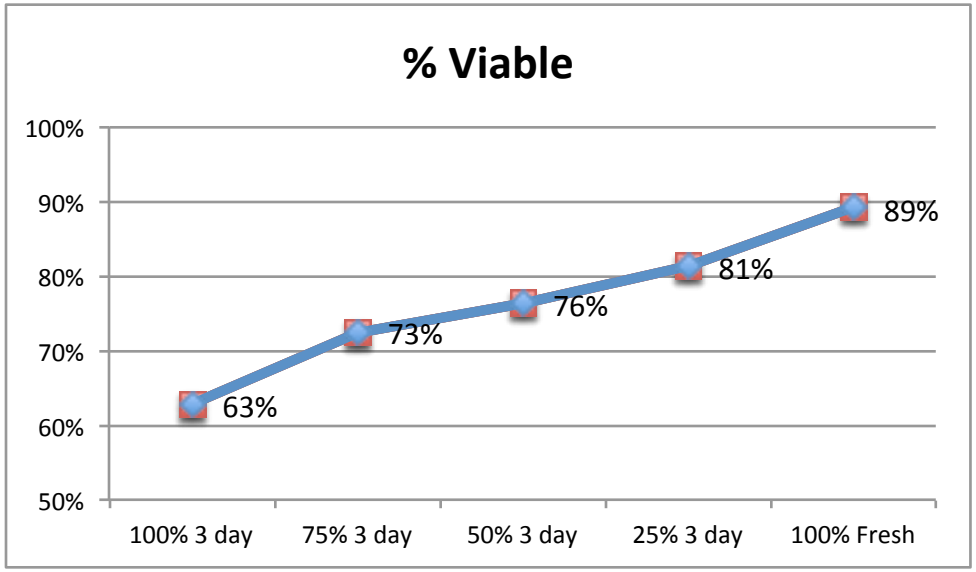
10/4/13

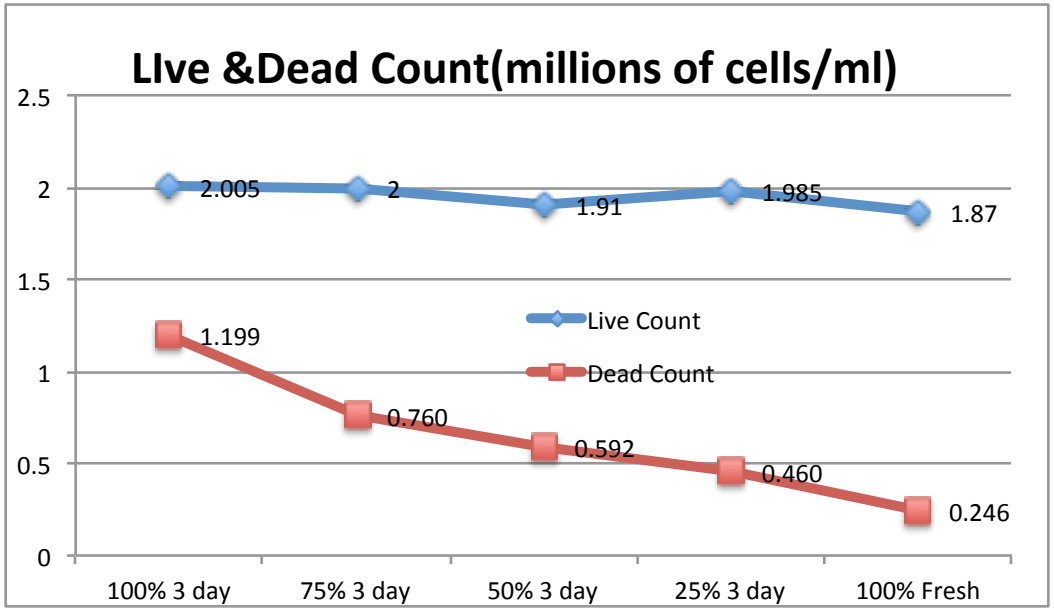
Sample Set Overview (test date: 20131004)
All prep performed by customer, PE CD4 Experiment

ORFLO Revolutionizing Life Science

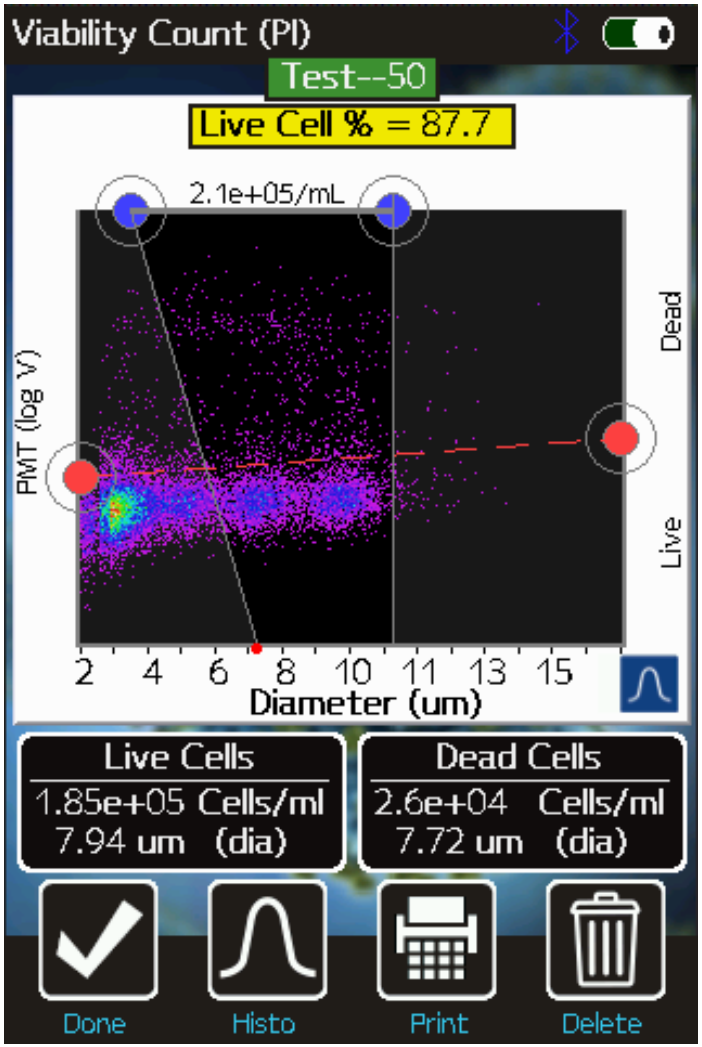
- Sample Cell Type: Peripheral Human Blood
- Sample Preparation:
  - Miltenyi Clinimax WBC isolation (?)
  - All 5 samples were mixed with Orflo's Moxi Cyte Viability reagent (15ul of sample to 135 ul of Moxi Cyte Viability reagent)
    - First tests were run after 5 minutes of incubation
    - Repeat was done 15-20 minutes later with some assumed negative impact on viability due to long incubation
  - Master sample 1 was pulled and thawed presumed to have high viability
  - Samples 2-5 were a mixture of sample 1 with 3 day old suspension culture of same cells
    - Sample 2 (test 50): 25% master to 75% 3 day suspension culture
    - Sample 3 (test 51): 50% master to 50% 3 day suspension culture
    - Sample 4 (test 53): 75% master to 25% 3 day suspension culture
    - Sample 5 (test 54): 100% 3 day suspension culture
  - Note test 52 was a blown test due to assumed reuse of cassette
  - Sample 6 was an isolated hematopoietic stem cell stained with standard stem cell marker (BD Pharmigen Primary PE anti human CD34 anti body catalog # xxxxxx, expired July 2012, with short 8 minute incubation and a brief wash step
  - Sample 7: 15 ul's o sample 6 mixed with 50 ul's of sample 1(?)

Charts (using average of the two repeats)

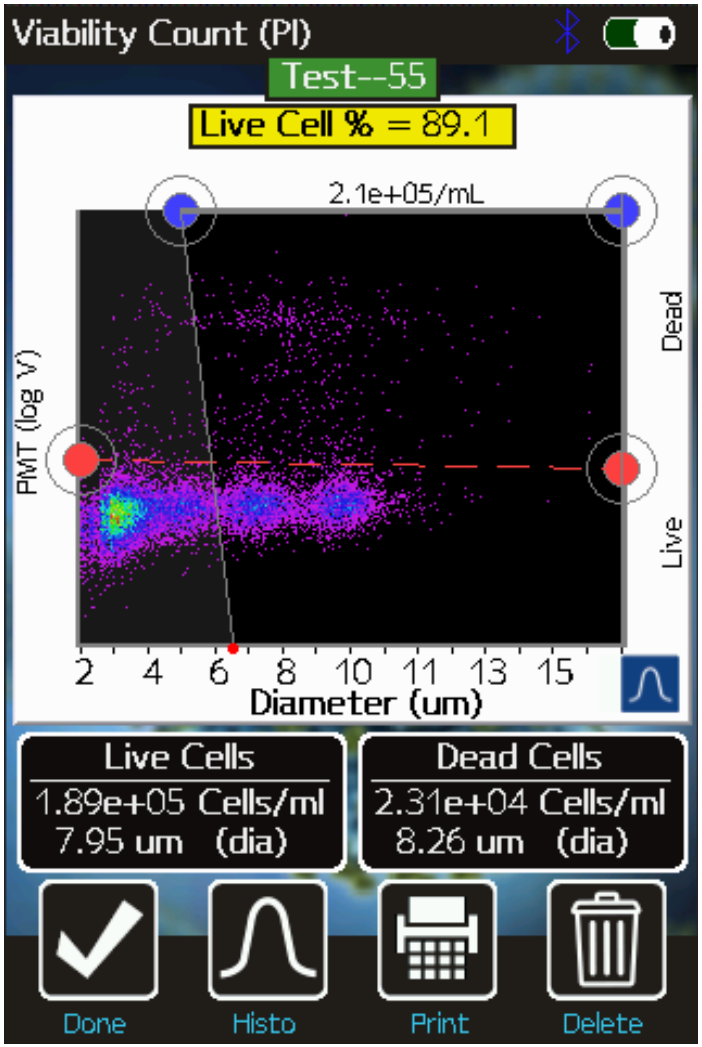




Sample 1 (100% Fresh sample)



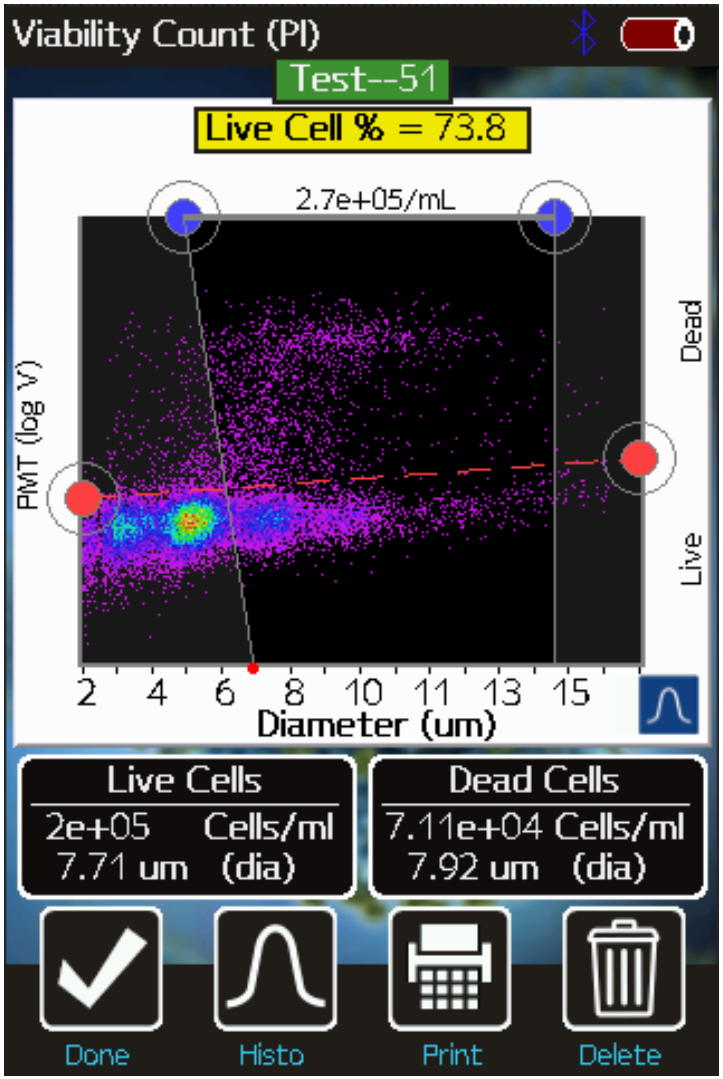
Test 50
t = (5 minutes post incubation



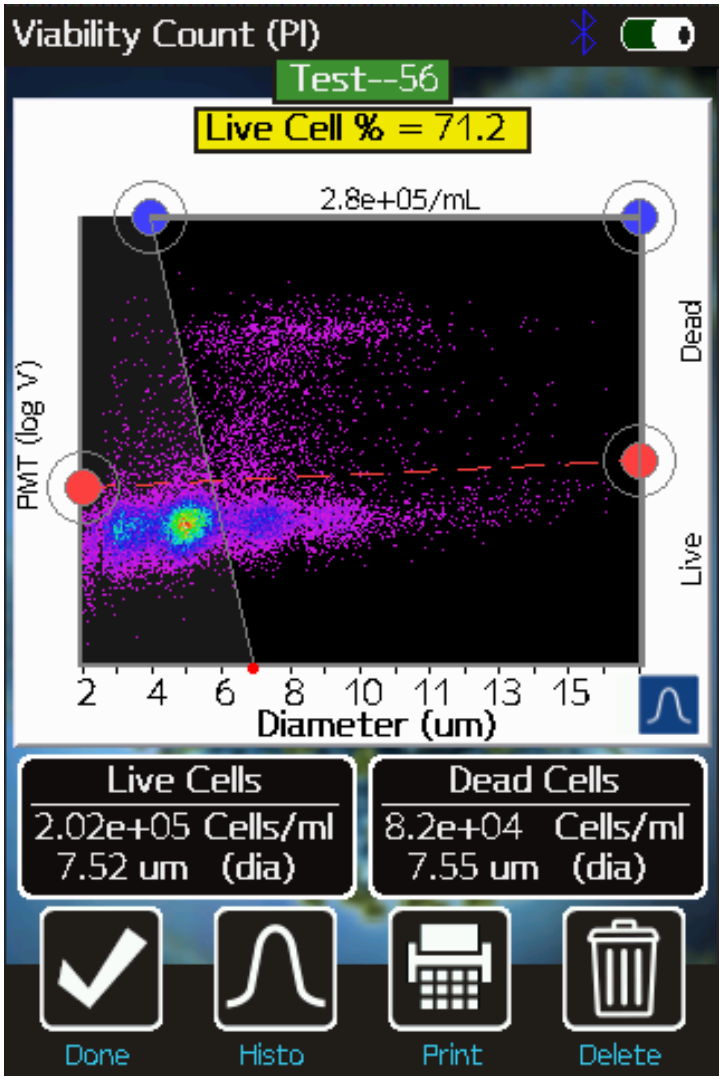
Run 55
t = ~ 12 minutes post incubation

Sample 2 (25% Fresh sample, 75% 3 day suspension culture)

ORFLO Revolutionizing Life Science



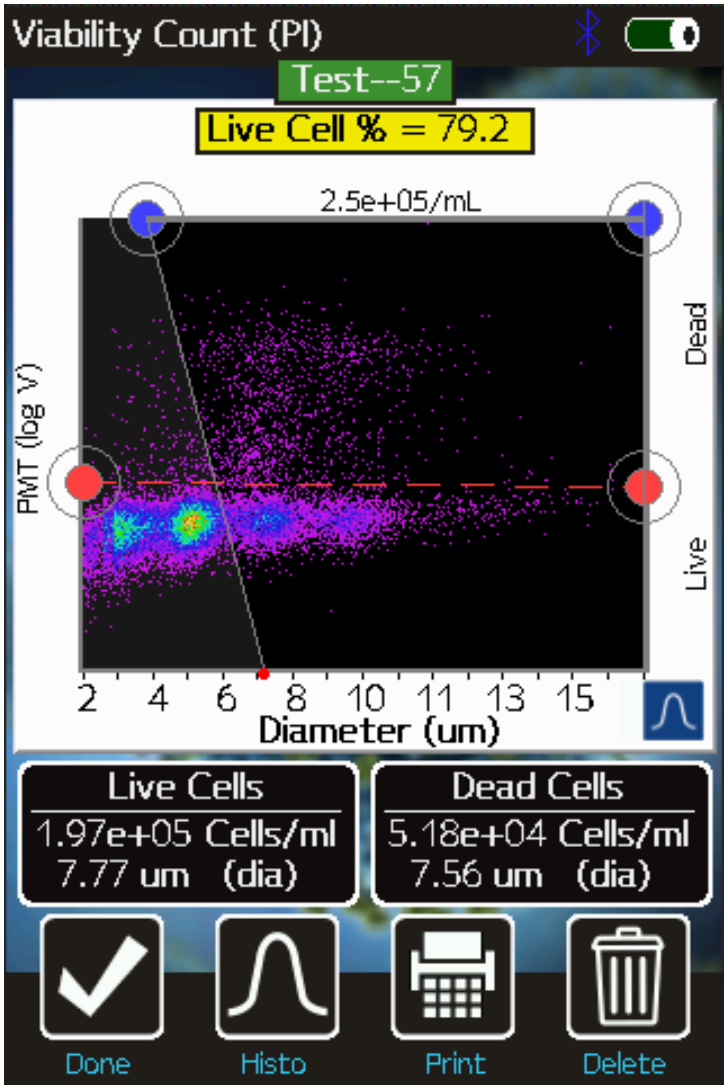
Test 51
t = ~ 5 minutes post incubation



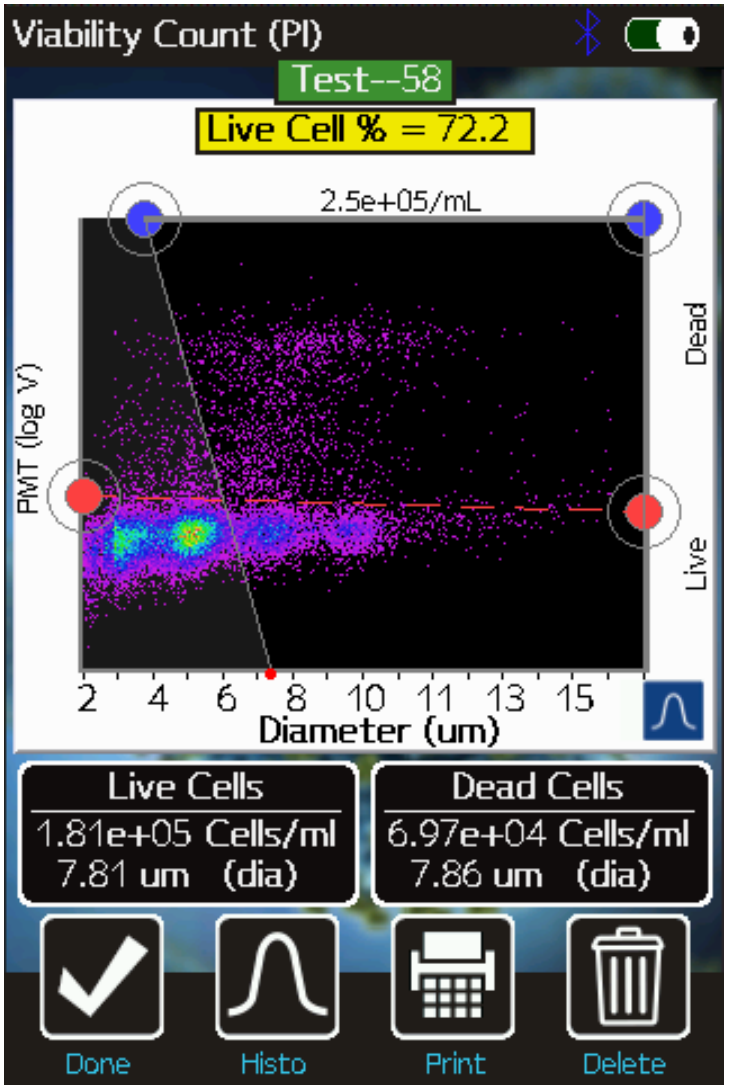
Test 56
t = ~ 12 minutes post incubation

Sample 3 (50% Fresh sample, 50% 3 day suspension culture)

ORFLO Revolutionizing Life Science

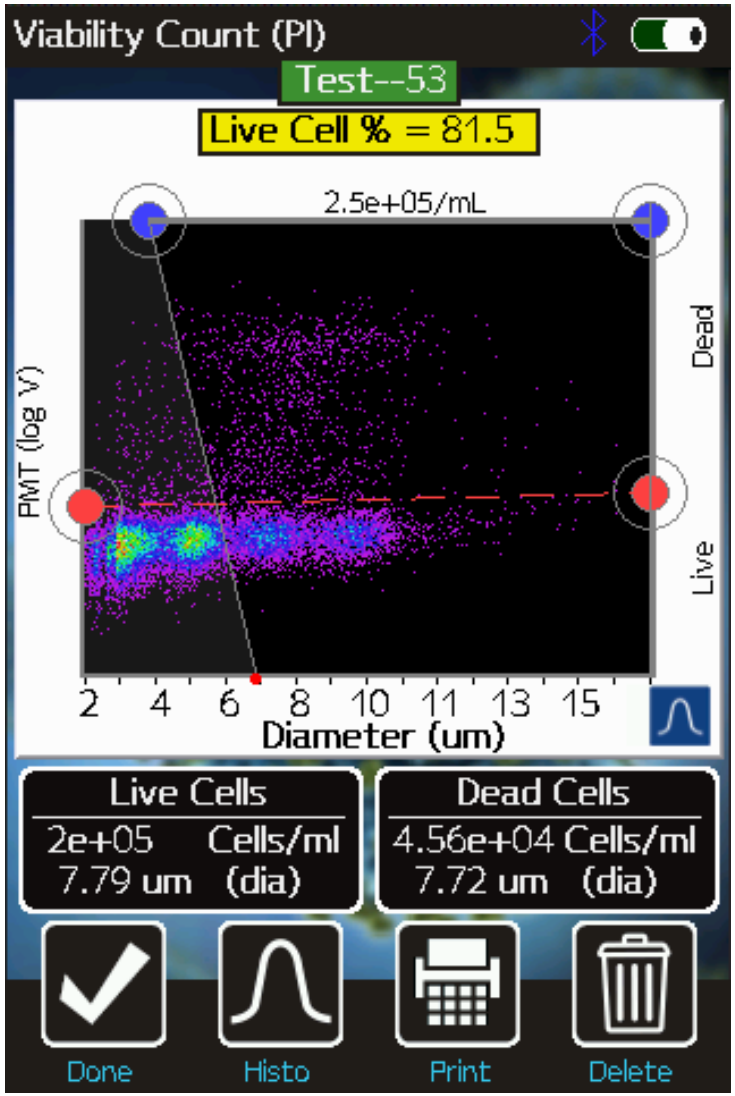


Test 57
t = ~ 13 minutes post incubation

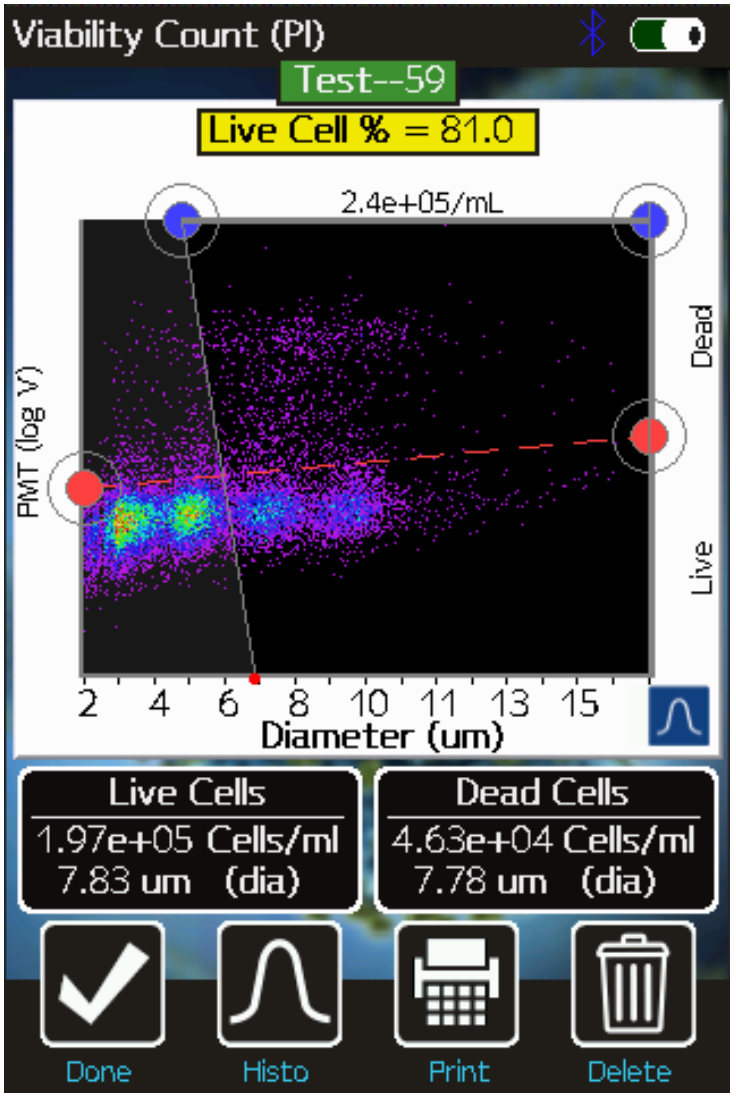


Test 58
t = ~ 13 minutes post incubation

Sample 4 (75% Fresh sample, 25% 3 day suspension culture)

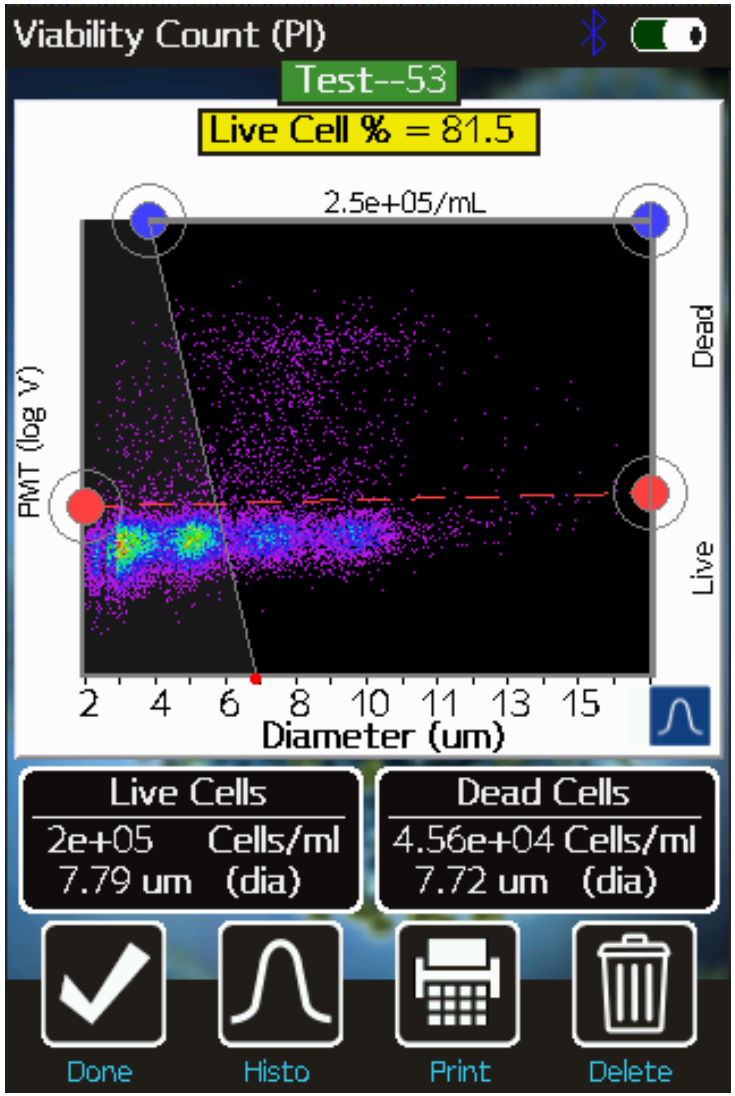


Test 53
t = ~ 6 minutes post incubation

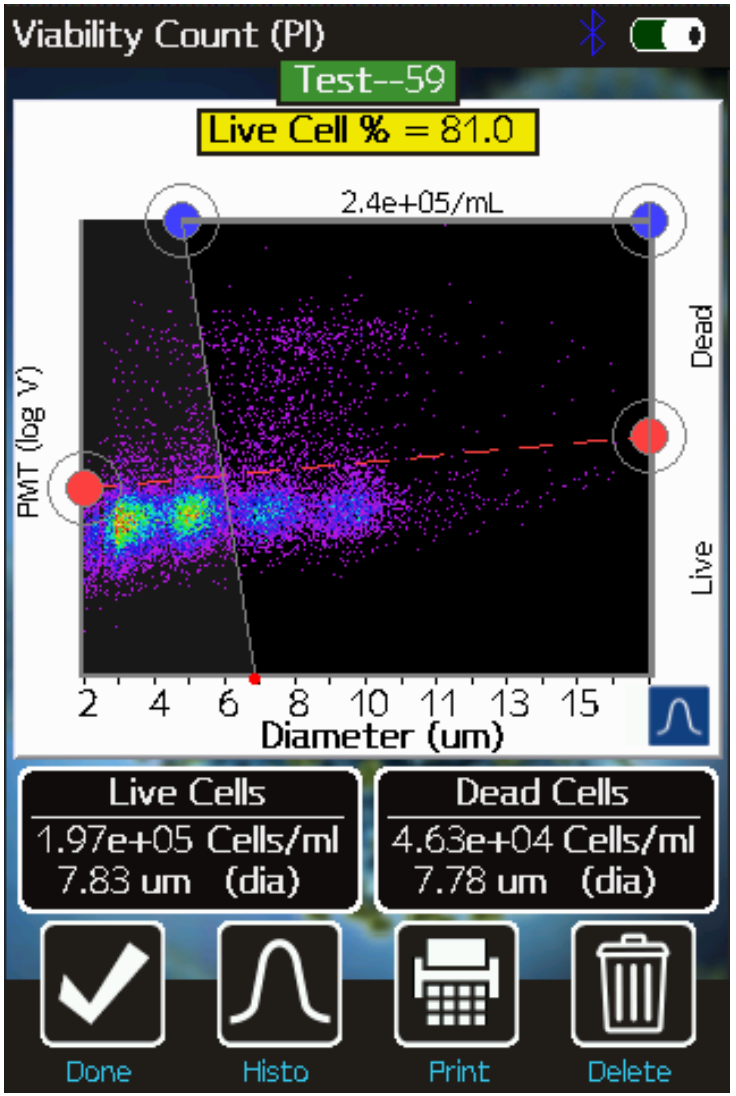


Test 59
t = ~ 13 minutes post incubation

Sample 4 (75% Fresh sample, 25% 3 day suspension culture)

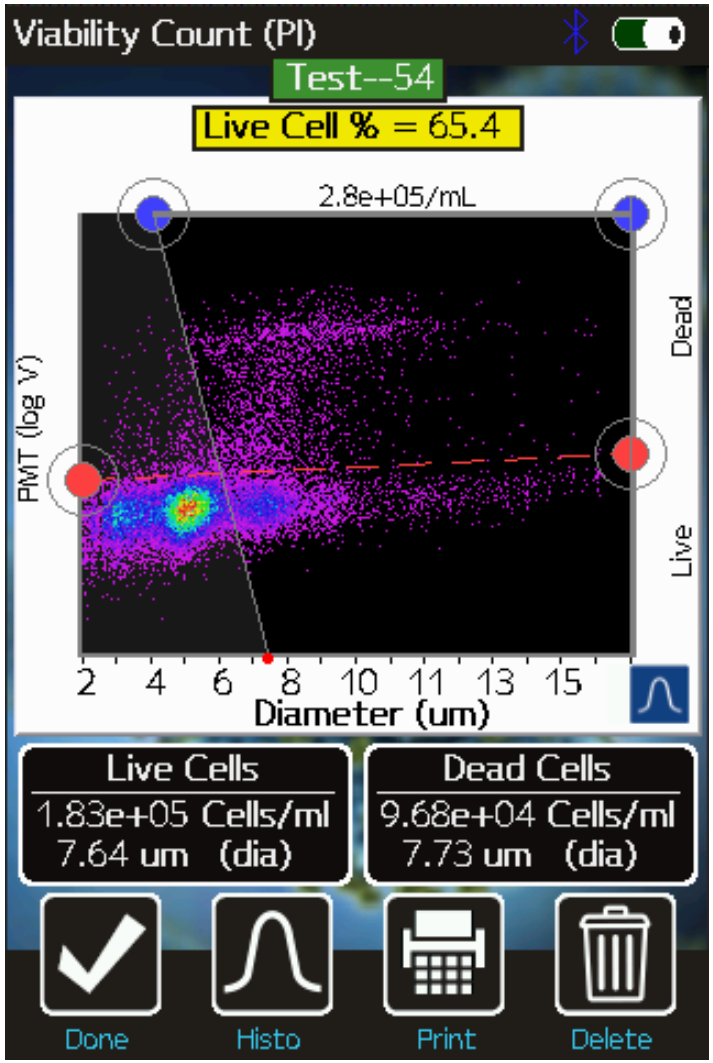


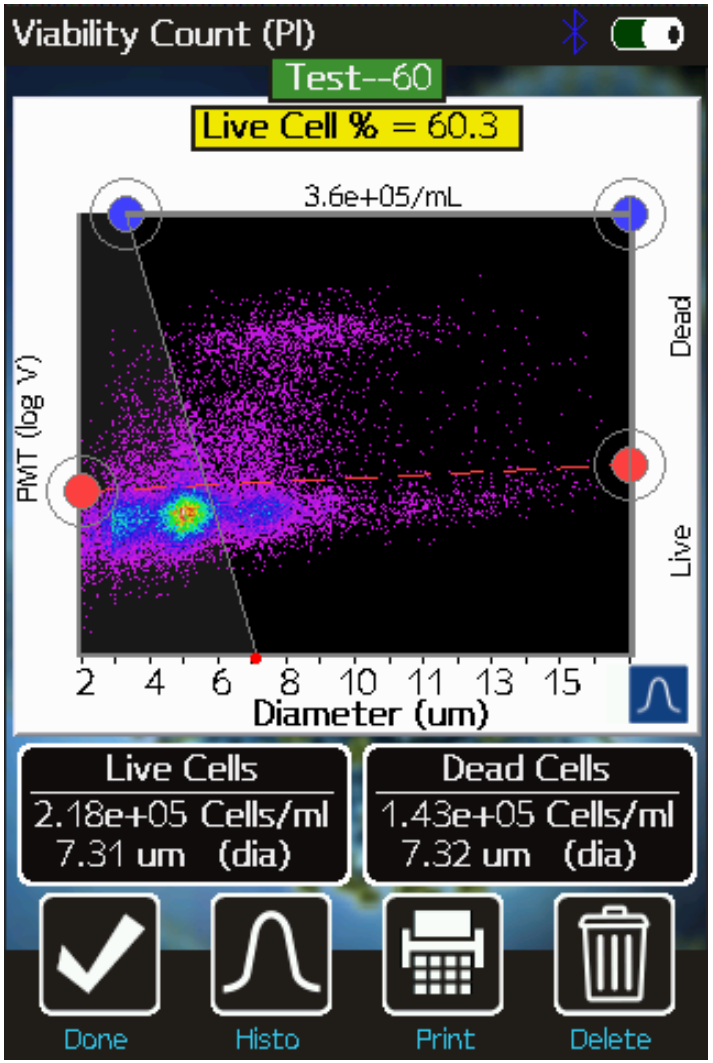
Test 53
t = ~ 6 minutes post incubation



Test 59
t = ~ 13 minutes post incubation

Sample 5 (100% 3 day suspension culture)



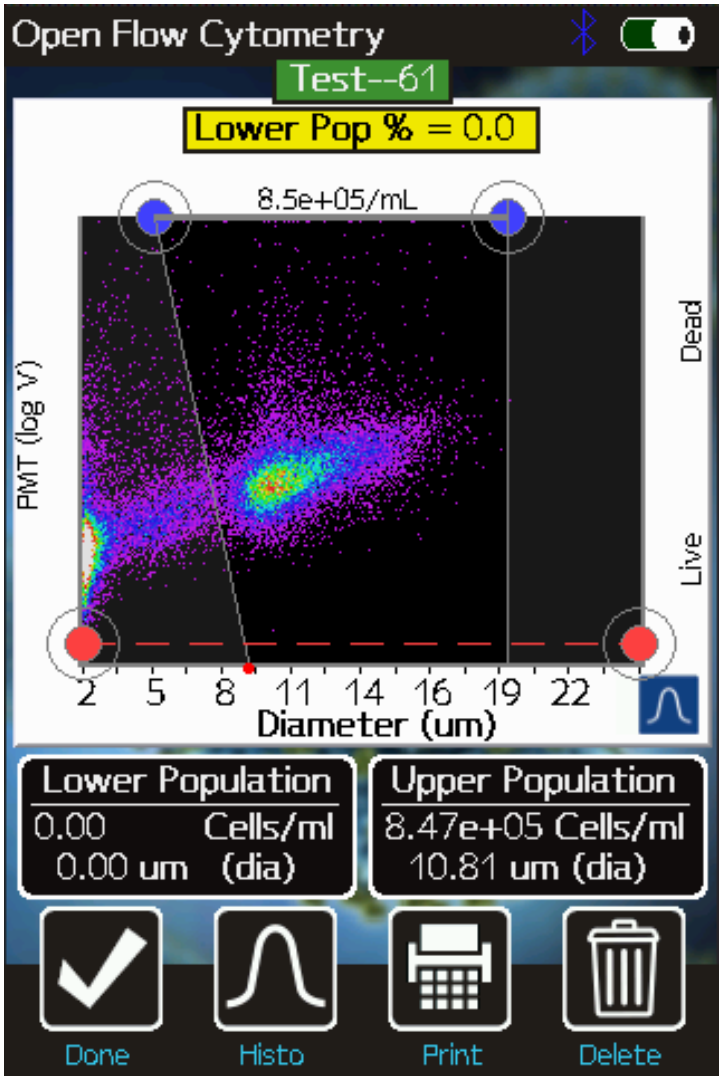


Test 54
t = ~ 6 minutes post incubation

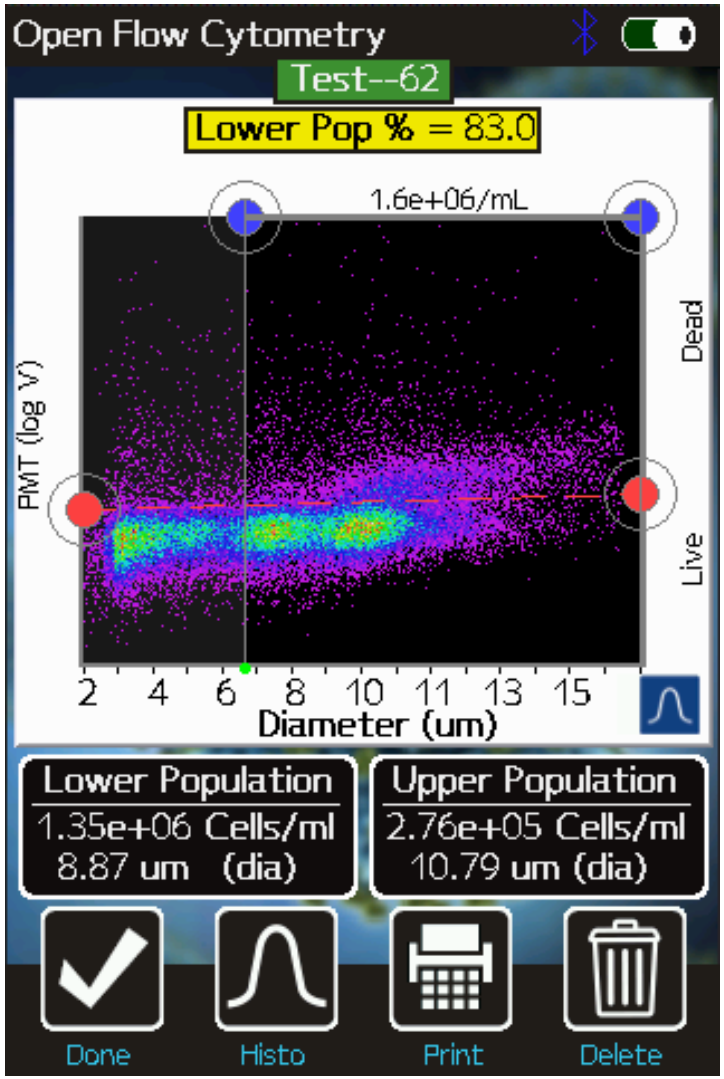
Test 60
t = ~ 14 minutes post incubation
Additional incubation lead to increase in cell death

Sample 6 & 7 (CD34 +)

ORFLO Revolutionizing Life Science



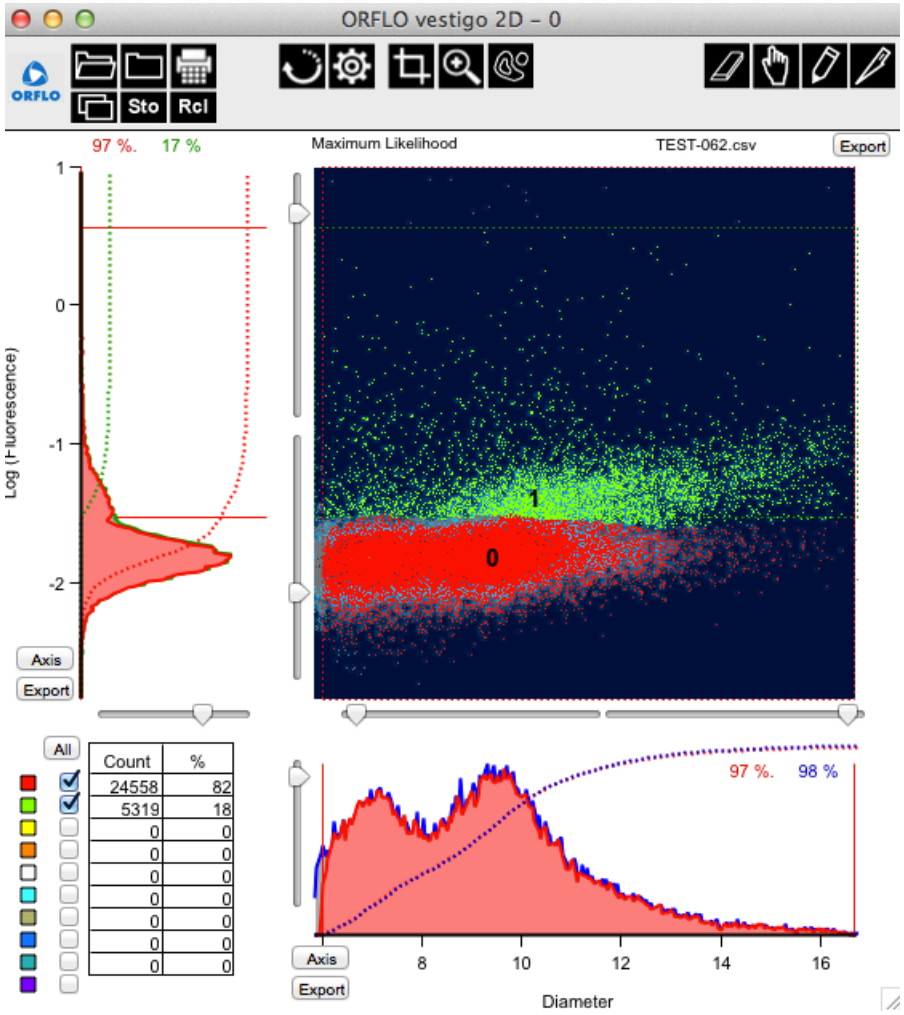
Test 61
PE Primary Ab Stain, CD34+



Test 62
Spiked CD34 stained population into non CD 34+ stained population

Sample 7 Data Analysis

Sample	Description	Total CD34 positive	Total CD34 negative	ul's of CD34 sample	ul's of fresh sample	CD34+ dilution	Expected CD34+ count from sample 6	Total actual measured volume by Moxi Flow (ul)	Absolute CD34- count Moxi Flow & VESTIGO	Absolute CD34+ count Moxi Flow & Vestigo	CD34- Cells/ml (millions) Moxi Flow and Vestigo	CD34+ Cells/ml (millions) Moxi Flow & VESTIGO	Measured CD34+ Error
6	PE C3 labeled sample	0.847	0	NA	NA	NA	NA						
7 (same as 1?)	Mixture	0.276		15	50	23%	0.195	27.5	24,558	5,319	0.893	0.193	1.05%



Conclusion:

- Moxi Flow with ORFLO's VESTIGO2D flow analysis software provides a highly accurate method for quantitating PE CD34+ Primary antibody stain
  - Note, antibody had expired in July 2012
- Shows about a 0.4 log separation which is sufficient to quantitate with ~1.5% error

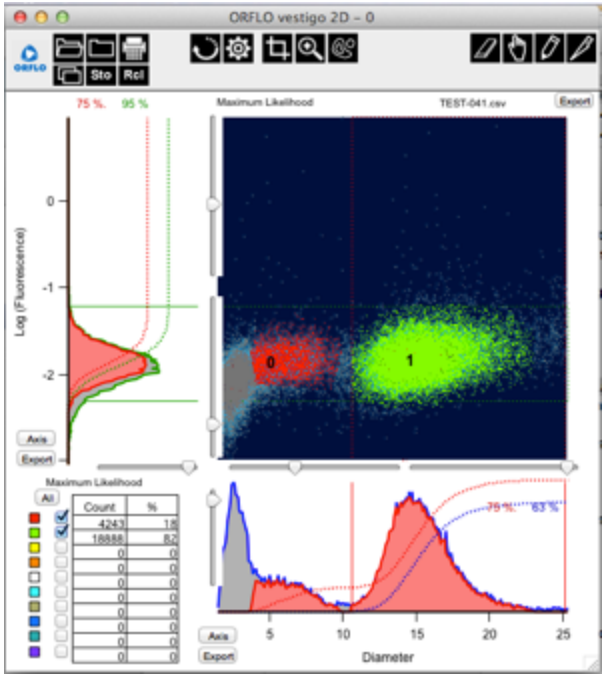
ORFLO Revolutionizing Life Science

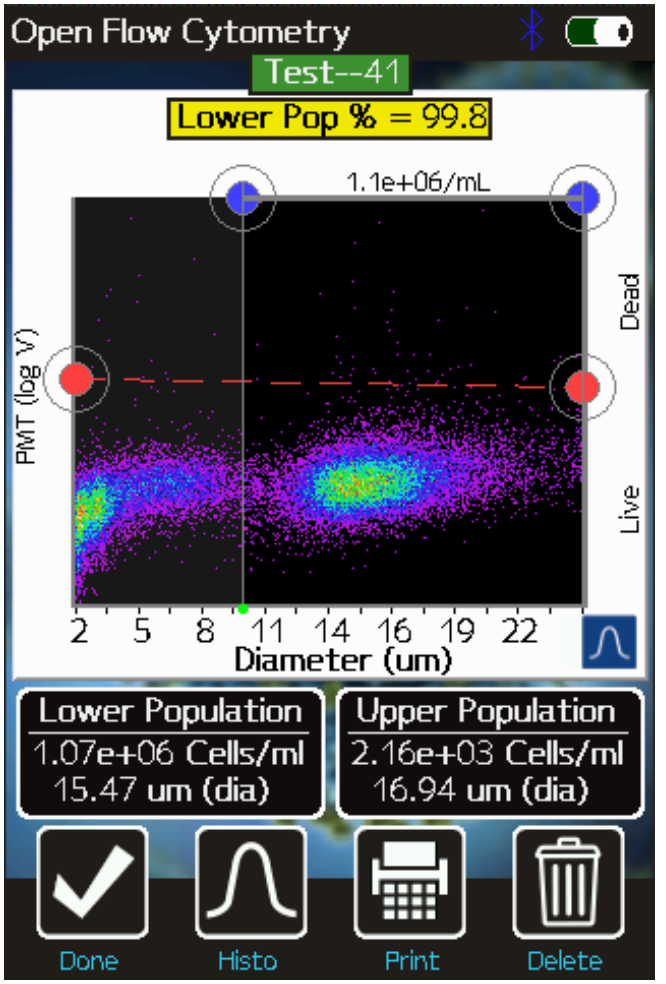
Moxi Flow Evaluation
Quantitating Transfection Efficiency

Using PE labeled anti human antibody to aVb3 (expressed on melanoma cells)

Biotech Customer, 9/25/13

Control, no stain



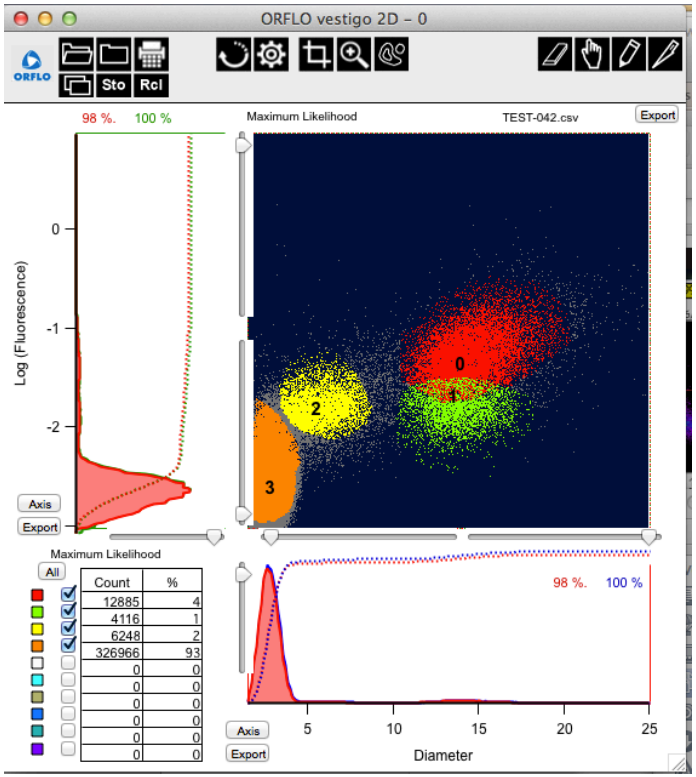


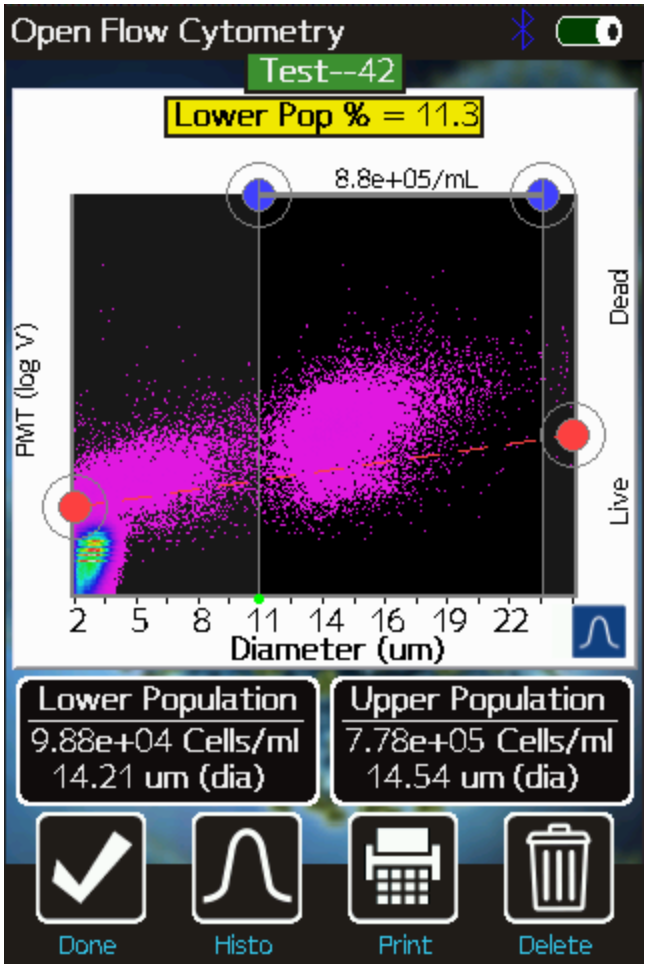
Comments

- Control, no PE anti human antibody to specific target
  - Not transfection
  - Stained with PE-anti-aVb3
  - Cell type: K562 (human erythroleukemia cell line)
- Cell concentration 1.06 x 10^ cells/ml
- Control population centered at -1.8 log (fluorescence)
- Red = debris
- Grey = system noise

Stained sample 1
Moxi Flow & Orflo's VESTIGO2D enables ***Instant*** quantification of transfection of efficiency

ORFLO Revolutionizing Life Science

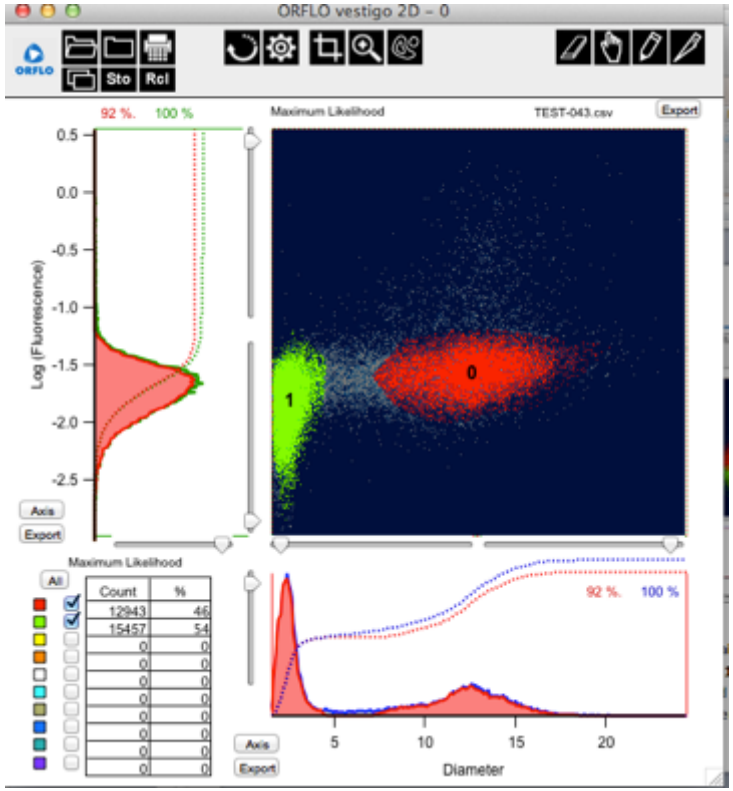


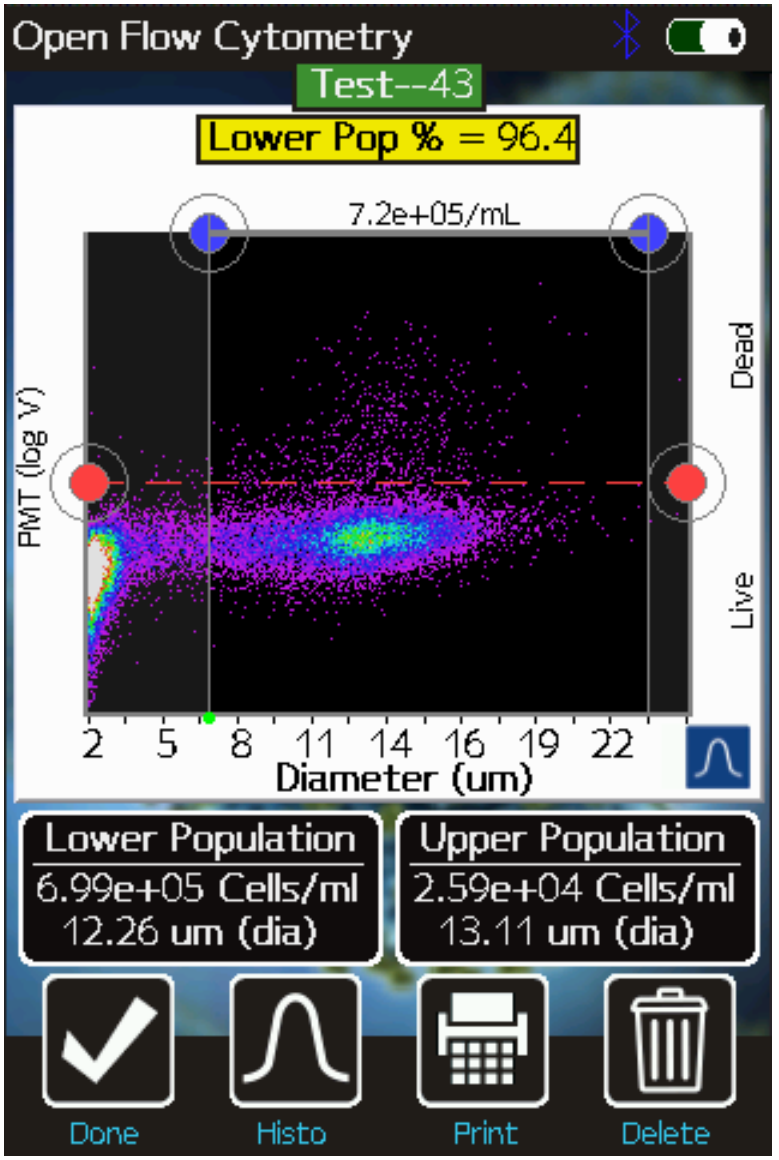


Comments

- Cell type: K562 (human erythroleukemia cell line)
  - Transfected with a human integrin aVb3 gen
  - Stained with PE-anti-aVb3
- Red = positive for stain (0.778M cells/ml), -1.3 log fluorescence (0.5 log separation from non-transfected population
- Green = Negative(0.1M cells/ml), -1.8 log fluorescence
- Yellow = non-stained debri population
- Orange = System noise

Control sample 2

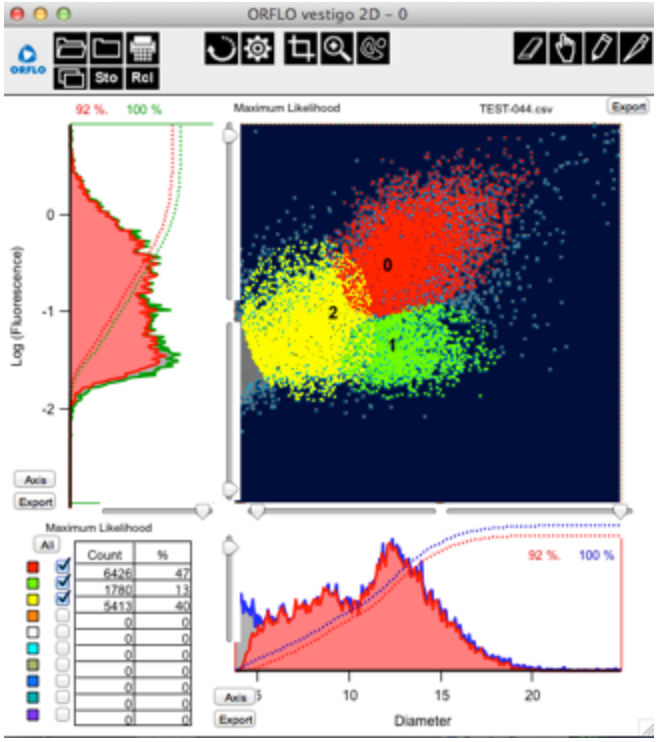




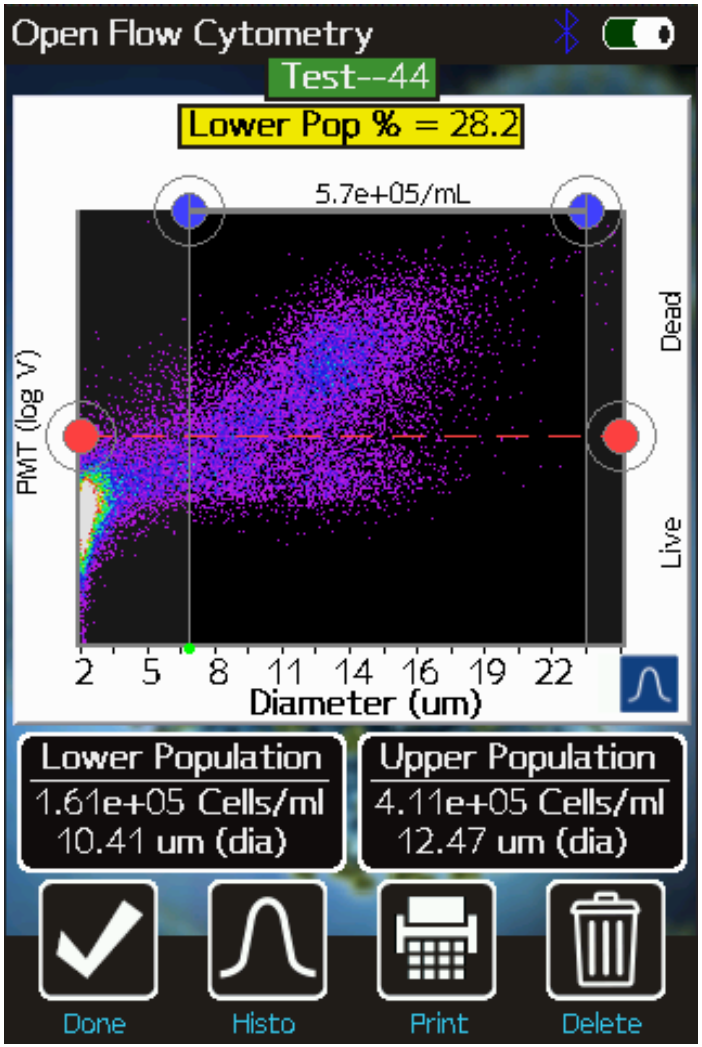
Comments

- Control, no PE anti human antibody to specific target
  - Not transfection
  - Stained with PE-anti-MICA
  - Cell Type = Ba/F3 mouse pro-B cell line
- Red = control population 0.7M cells/ml (centered at -1.6 log of fluorescence)
- Green = noise
- Grey = debris

Control sample 2
Moxi Flow & Orflo's VESTIGO2D enables ***Instant*** quantification of transfection of efficiency



Sample Snapshot	Count	%
+ Transfected Cells (red)	6,426	59%
Non transfected cells (green)	1,780	21%
Unknown cell population (yellow)	5,413	19%



Comments

- Control, no PE anti human antibody to specific target
  - Transfected with human MICA
  - Stained with PE-anti-MICA
  - Cell Type = Ba/F3 mouse pro-B cell line
- Green = negative population (1,780 cells), centered at -1.5 log of fluorescence
- Red = positive population, centered at -0.4 log of fluorescence
- Yellow (unknown population)
- Grey = cell debris

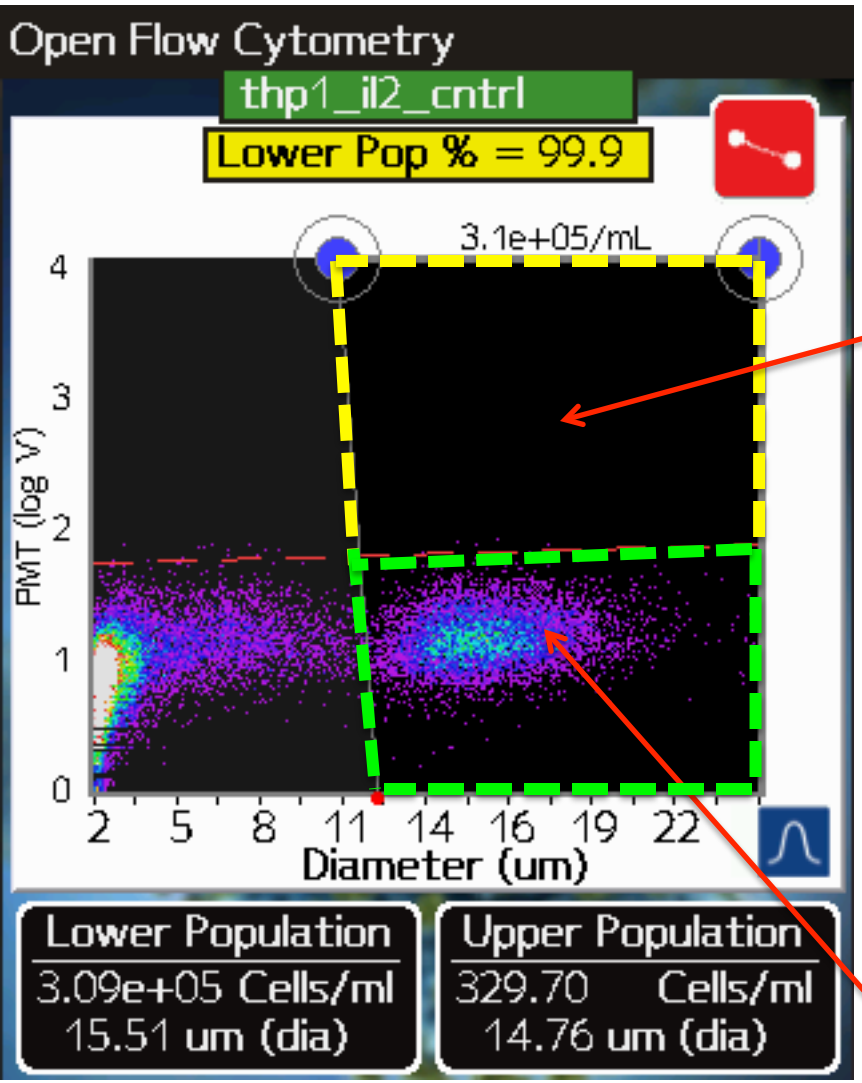
ORFLO Revolutionizing Life Science

Moxi Flow Evaluation
Quantitating IL2 & TLR2 Expression

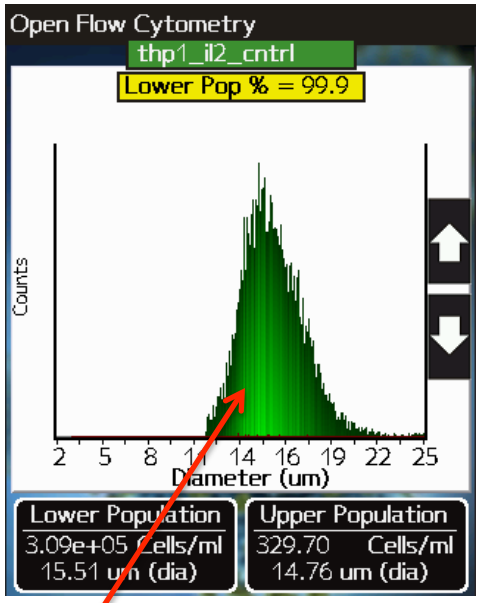
Using PE labeled anti human antibody to IL2 & TLR2

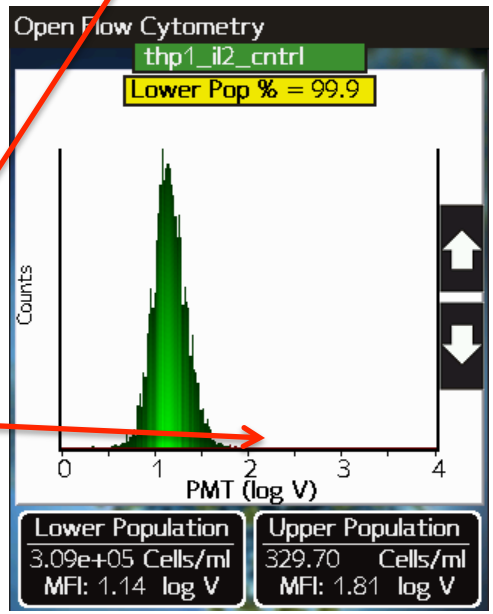
UCSD, 11/08/13

Mouse THP1 Cell, CONTROL, no staining

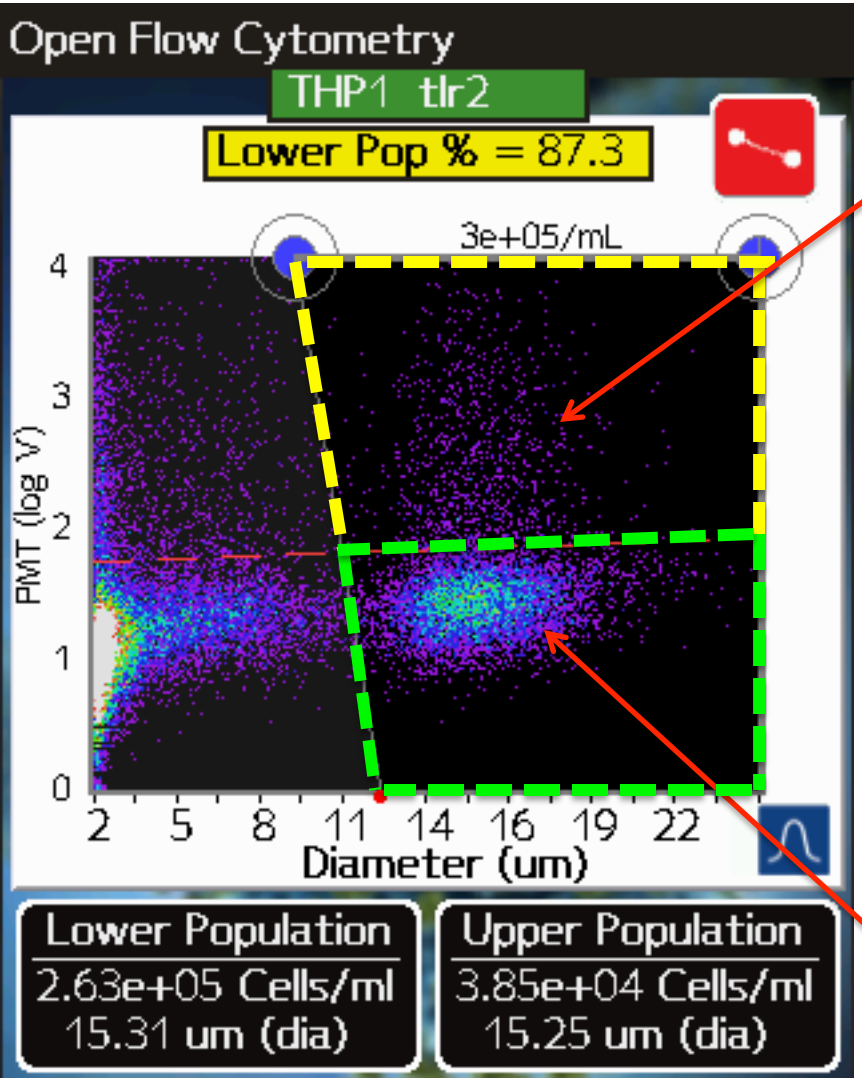


- These are the gated regions
- + cells (0.0%)
- All values for fluorescence intensity and size will be generated from events only occurring in the these two regions
- The brighter the population, the more concentrated, in other words this is a concentration heat-map
- X-axis = direct size
- Y-axis = fluorescence
- Negative or unstained cells (309K/ml), 15.5um mean diam)

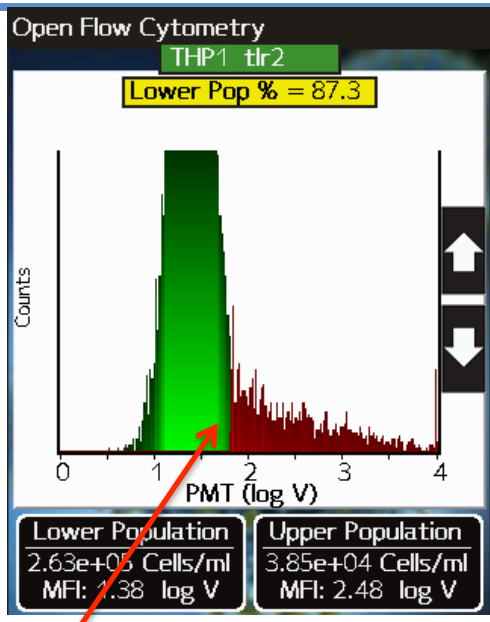


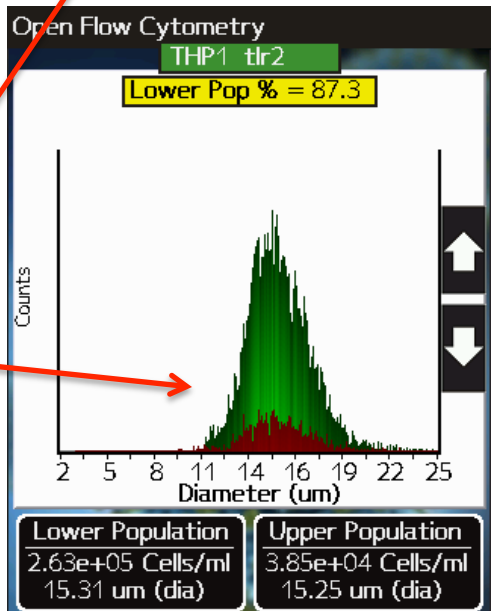


Mouse THP1 Cell, PE labeled anti-mouse TLR2 (CD2 282)

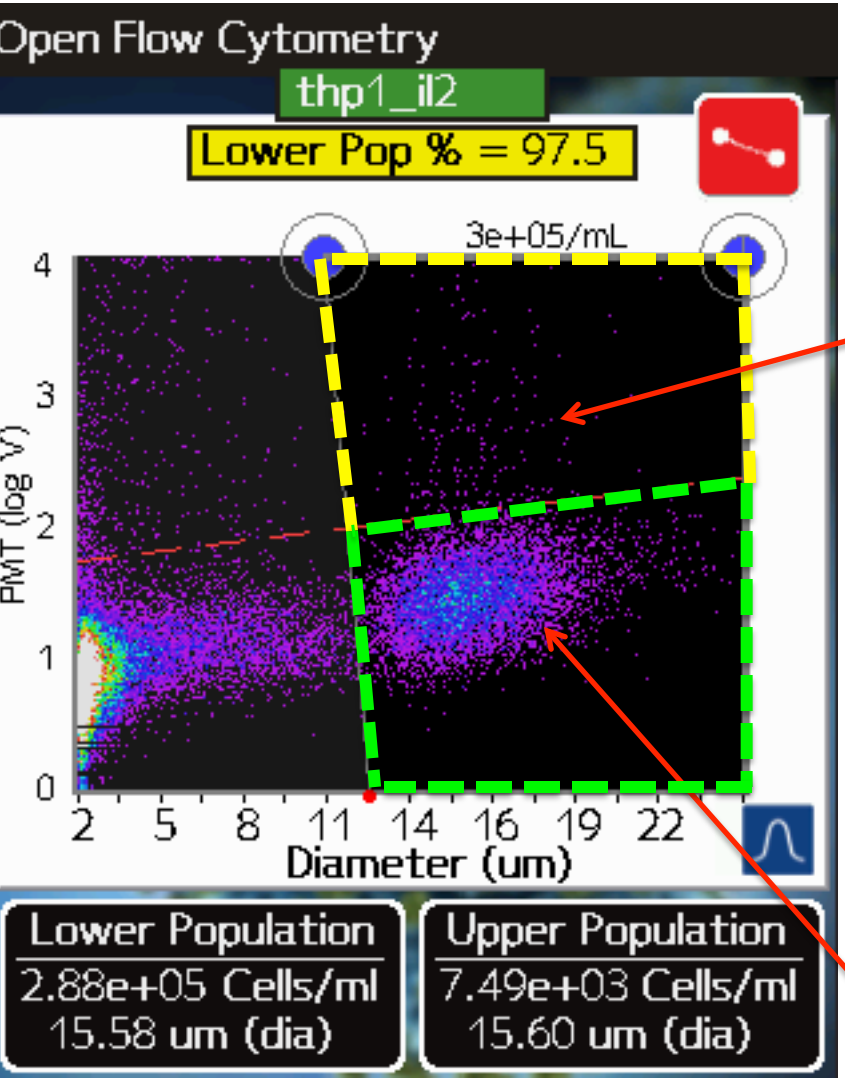


- These are the gated regions
- + cells (12.7% stained +)
- All values for fluorescence intensity and size will be generated from events only occurring in the these two regions
- The brighter the population, the more concentrated, in other words this is a concentration heat-map
- X-axis = direct size & morphology w/ 10nm resolution
- Y-axis = fluorescence
- Negative or unstained cells

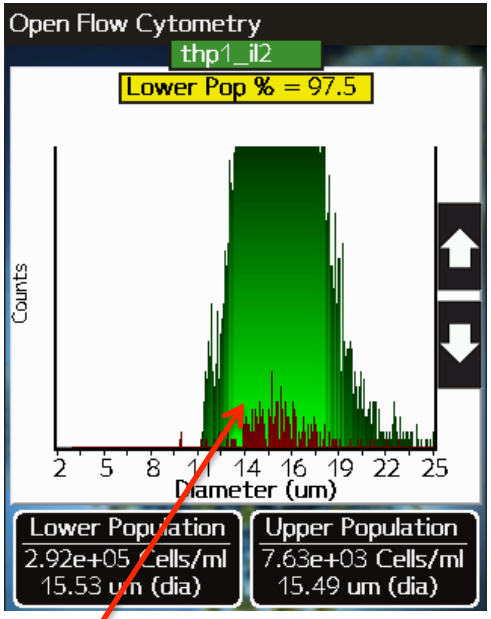


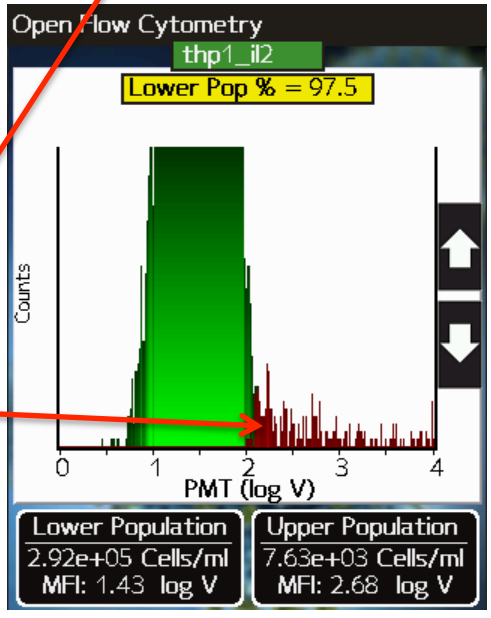


Mouse THP1 Cell, PE labeled anti-mouse IL2 (CD 122)



- These are the gated regions
- + cells (2.5% stained +)
- All values for fluorescence intensity and size will be generated from events only occurring in the these two regions
- The brighter the population, the more concentrated, in other words this is a concentration heat-map
- X-axis = direct size
- Y-axis = fluorescence
- Negative or unstained cells





ORFLO Revolutionizing Life Science

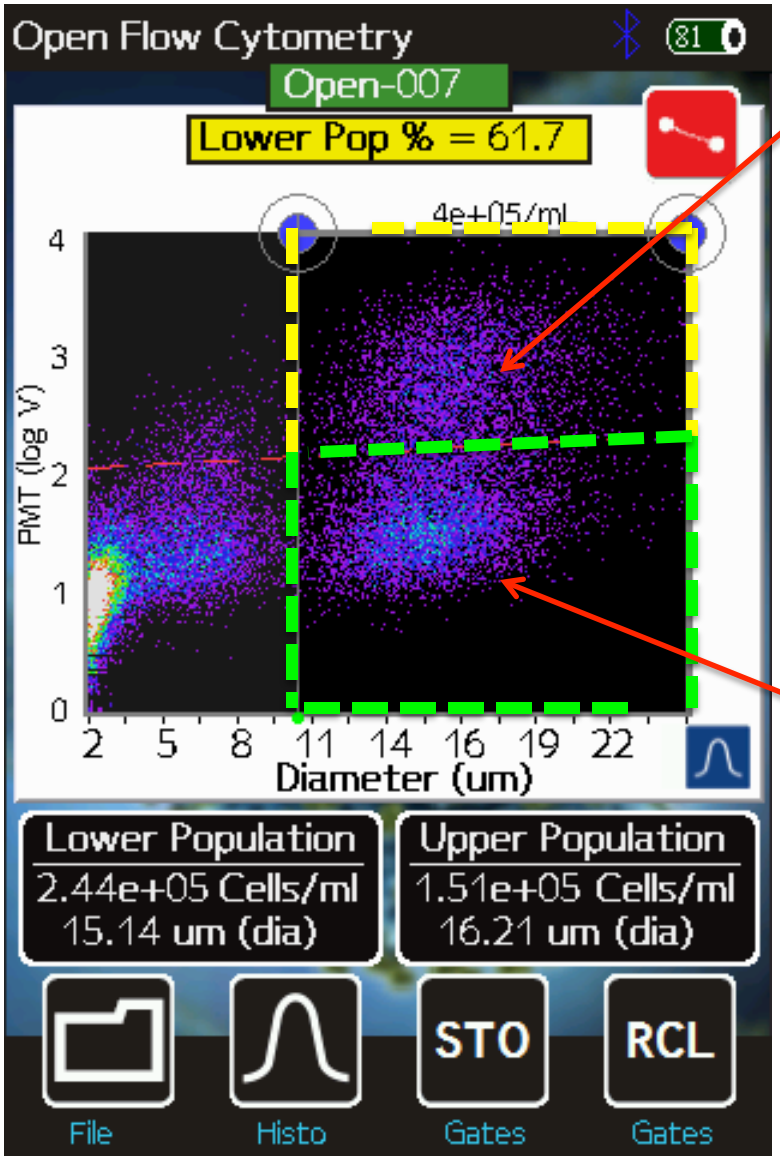
Moxi Flow Evaluation Quantitating ??

Using PE labeled anti human antibody to ??

Scripps Lab

11/11/13

?? Cell, PE Labeled anti XX to XX



- These are the gated regions
- + cells (38.3%, 151K cells/ml, 16.21 mean diameter)
- All values for fluorescence intensity and size will be generated from events only occurring in the these two regions
- The brighter the population, the more concentrated, in other words this is a concentration heat-map
- X-axis = direct size
- Y-axis = fluorescence
- Negative or unstained cells (61.7%, 244K/ml, 15.14um mean diameter)
- Note the 1um shift in size for the stained cells